

## ABSTRACT

Title of Document:

**PROMOTING THE PRODUCTION AND  
CONSUMPTION OF WHEAT-BASED  
FUNCTIONAL FOODS RICH IN  
ANTIOXIDANTS**

*Marla West Luther, Doctor of Philosophy, 2008.*

Directed By:

Associate Professor Liangli (Lucy) Yu  
Department of Nutrition and Food Science

The present study evaluated the effects of solid state yeast treatment and thermal processing on the extractability and in-vitro bioavailability of antioxidants of wheat bran and whole-wheat pizza crusts, and developed possible sample outreach materials for promoting the consumption on whole-wheat functional foods. The first section of this research analyzed the effect of solid-state yeast treatment on the extractable antioxidant properties of wheat bran. Wheat bran was treated with ten commercially available food grade yeasts at two doses under solid state conditions. Antioxidant properties were evaluated by measuring total phenolic content, and radical scavenging capacities against the cation ABTS, peroxy (ORAC), DPPH (RDSC), and hydroxyl (HOSC) radicals. Results showed that under the selected conditions, yeast strain LBE.11 was able to increase ORAC and HOSC radical scavenging by 50% and 67%, respectively.

The second part of this study evaluated the impact of bran particle size, fermentation time, and baking time and temperature on the 100% ethanol extractable antioxidant capacities of whole wheat pizza crust. Results showed that pizza crusts produced with reduced particle size bran maintained antioxidant capacity throughout thermal processing. At 18 hrs of fermentation RDSC and TPC values of the pizza crusts were increased by 17% and 23%, respectively. Results showed that increasing baking temperature increased RDSC values by 14 and 17% for pizza crusts prepared with Lakin and Trego variety wheat, respectively. The third part of this study analyzed the in-vitro bioavailability of wheat antioxidants in the baked pizza crusts. The antioxidant properties were evaluated by measuring total phenolic content, and radical scavenging capacities against the cation ABTS, peroxy (ORAC), DPPH (RDSC), and hydroxyl (HOSC) radicals. Results showed that increasing baking temperature increased the in-vitro bioavailable TPC by up to 54%. Pizza crusts with higher available antioxidants may have a greater level of bio-available antioxidants.

In the final segment of this research a tri-fold was developed for promoting the consumption of whole wheat foods. The tri-fold was designed to include food-specific knowledge and consumption consequences knowledge in addition to recipes, directions, and nutritional facts. Integrating research findings into outreach materials may be an effective way to increase functional food consumption. Results from these studies indicate that there are numerous ways to increase antioxidant levels in the diet.

**PROMOTING THE PRODUCTION AND CONSUMPTION OF WHEAT-  
BASED FUNCTIONAL FOODS RICH IN ANTIOXIDANTS**

By

Marla West Luther

Dissertation submitted to the Faculty of the Graduate School of the  
University of Maryland, College Park, in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
2008

Advisory Committee:  
Professor Liangli (Lucy) Yu, Chair  
Professor Thomas Castonguay  
Professor Mickey Parish  
Professor David Lei  
Dr. Jun-Jie Yin

© Copyright by  
Marla W. Luther  
2008

## Acknowledgements

This achievement is the result of kindness, encouragement, direction, and support from peers, instructors, friends, and family. Thank you to my fellow labmates, in particular, Kequan (Kevin) Zhou, John Parry, Jeff Moore, Zhihong Cheng, and Margaret Slavin who have assisted me throughout my graduate experience at the bench as well as insight into new solutions and ideas. You not only gave of your time and knowledge, but created a friendly environment that I have enjoyed working and learning in. Dr. Liangli (Lucy) Yu, thank you for giving me this opportunity and for your guidance and assistance. I would also like to thank my committee members for their assistance and support in completing my graduate work. Dr. Thomas Castonguay, Dr. David Lei, Dr. Mickey Parish, and Dr. Jun-Jie Yin. My heart-felt gratitude also goes out to my family for helping me to keep things in perspective and laugh often. I would be remiss not to thank my parents Lonnie and Mina Luther by name for their sound guidance, unselfish love, unconditional support, and continual prayers.

## Table of Contents

Acknowledgements.....	ii
List of Tables .....	v
List of Figures .....	vi
Wheat as a Commodity .....	1
Wheat Consumption.....	1
Wheat Fractions .....	2
Wheat Flours .....	2
Health Promoting Properties of Whole Grains .....	5
Epidemiological Studies .....	5
Dietary Fiber .....	6
Antioxidant Activity & Energy Balance.....	9
Government Approved Health Claims.....	11
Whole Wheat Product Market .....	12
Functional Foods.....	13
Functional Foods and Nutraceuticals.....	13
Functional Food Outlook .....	13
Nutraceutical Components.....	15
Antioxidants.....	17
Antioxidants and Human Health.....	17
Methods of Antioxidant Analysis .....	19
Wheat Antioxidants .....	19
Antioxidants in Wheat Fractions .....	19
Effect of Storage & Processing on Wheat Antioxidants.....	20
Antioxidants in Wheat Based Food Products .....	21
Effect of Solid State Reactions on Antioxidants.....	24
Solid State Reactions .....	24
Nutritional Outreach .....	25
Outreach Development Tools .....	25
U.S. Food Assistance Programs: Education & Nutrition.....	27
Impact of Nutrition Knowledge on Health .....	33
Food, Economics & Health.....	34
Literature Cited .....	35
Chapter 2: Effect of Yeast Strain on the Antioxidant Properties of Wheat Bran	
Following Solid-State Treatments .....	48
Abstract .....	48
Introduction.....	48
Materials and Methods.....	51
Results and Discussion .....	57
Literature Cited .....	65
Chapter 3: Effects of Thermal Treatment, Fermentation, and Bran Particle Size on the	
Ethanol Extractable Antioxidant Properties of Whole-Wheat Pizza Crust .....	68
Abstract .....	68
Introduction.....	69

Materials and Methods.....	70
Results and Discussion .....	76
Conclusion .....	91
Literature Cited .....	92
Chapter 4: In-vitro Bioavailability of Wheat Antioxidants in Baked Pizza Crust.....	95
Abstract .....	95
Introduction.....	96
Materials and Methods.....	98
Results and Discussion .....	105
Conclusion .....	110
Literature Cited .....	111
APPENDIX A. Yeast Log Growth Curve* .....	113
Chapter 5: Development of a Tri-fold to Increase Whole Wheat Pizza Crust	
Consumption in College Students.....	114
Abstract .....	114
Introduction.....	114
Tri-fold Development .....	117
Pizza Recipes .....	120
Health Claims.....	120
Conclusion .....	121
Literature Cited .....	122

## List of Tables

**Table 1.1** New product introductions with a “whole grain” claim.

**Table 3.1** Effect of Bran Mesh Size on Pizza Crust Antioxidant Properties for Two Hard Wheat Varieties

**Table 3.2** Effect of Fermentation Time on Pizza Crust Antioxidant Properties for Two Hard Wheat Varieties

**Table 4.1** Fed-state synthetic digestive fluid composition



## List of Figures

- Figure 1.1** U.S. Per Capita Consumption by Disappearance (USDA 2008).
- Figure 1.2** U.S. Weighted Average Farm Price per Bushel (USDA 2008).
- Figure 1.3** Macronutrient Comparison of King Arthur Commercially Available Flours (King Arthur 2008).
- Figure 1.4** Effects of enzyme treatment on antioxidants in wheat bran.
- Figure 2.1** Effect of solid state yeast treatment on TPC of soft red wheat bran.
- Figure 2.2** ABTS•+ scavenging by solid state yeast treated soft red wheat bran.
- Figure 2.3** Effect of solid state yeast treatment on ORAC of soft red wheat bran.
- Figure 2.4** Effect of solid state yeast treatment on HOSC of soft red wheat bran.
- Figure 2.5** Effect of solid state yeast treatment on the DPPH scavenging capacities of soft red wheat bran.
- Figure 3.1** Effect of thermal treatment on the TPC of whole wheat pizza crust.
- Figure 3.2** Effect of thermal treatment on ABTS•+ of whole wheat pizza crust.
- Figure 3.3** Effect of baking time and temperature ORAC capacities of whole wheat pizza crusts.
- Figure 3.4** Effect of baking time and temperature on the DPPH scavenging capacities of whole wheat pizza crusts.
- Figure 3.5** Effect of Baking Time and Temperature on Soluble Free Ferulic Acid of Whole Wheat Pizza Crusts.
- Figure 3.6** Effect of Baking Time and Temperature on Soluble Conjugated Ferulic Acid of Whole Wheat Pizza Crusts.
- Figure 3.7** Effect of Baking Time and Temperature on Insoluble Bound Ferulic Acid of Whole Wheat Pizza Crusts.
- Figure 4.1** In-vitro Digestion.
- Figure 4.2** In-vitro bioavailable total phenolic contents (TPC) of baked pizza crusts.
- Figure 4.3** In-vitro bioavailable ABTS•+ scavenging capacities of baked pizza crust.
- Figure 4.4** In-vitro bioavailable ORAC scavenging capacities of baked pizza crust.
- Figure 4.5** In-vitro bioavailable HOSC scavenging capacity of antioxidants from whole wheat pizza crust.

**Figure 5.1** Hierarchy of Knowledge, Psychomotor Skill, and Food Related Sensory Attributes Leading to Increased Consumption of Functional Foods.

**Figure 5.2** Functional Whole Wheat Pizza Tri-fold – Side A

**Figure 5.3** Functional Whole Wheat Pizza Tri-fold – Side B

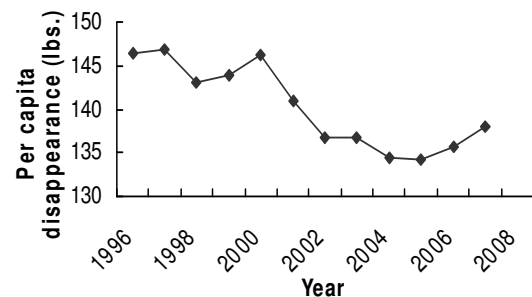
## Chapter 1: Literature Review

### Wheat as a Commodity

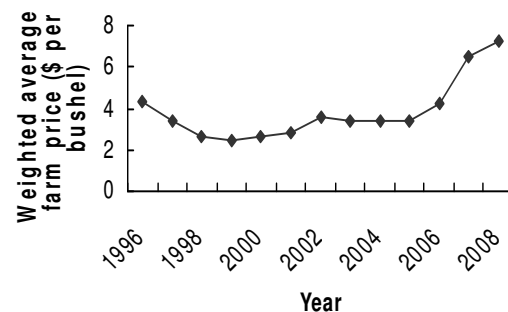
#### Wheat Consumption

Wheat (*Triticum spp.*) was one of the first crops to be cultivated. A global dietary staple, wheat is an important cereal grain in most temperate zones and has been adapted to many types of cuisine. Wheat comprises one-third of the total grain crop world wide (Slavin 2001). U.S. consumption of wheat based products is 117.5 grams per day, over 70% of the total grain intake (USDA 2007). Consumption of corn, rice and oats followed at 27, 17.9 and 3.05 grams per day, respectively. Barley and rye are each consumed at less than 0.5 grams per day (USDA 2007).

Multiple factors including diet trends and price have impacted wheat consumption. One pound of wheat produces approximately 0.98 pound of whole-wheat flour but only 0.74 pound of refined flour (USDA 2006). Per capita U.S. wheat consumption (Figure 1.1) is currently rising after experiencing fluctuations peaking in 1997 at 146.8 lbs. per person and dropping to 134.2 lbs. per person in 2005. Meanwhile, contrast cost of wheat production has increased and the weighted-



**Figure 1.1:** U.S. Per Capita Consumption by Disappearance (USDA 2008)



**Figure 1.2:** U.S. Weighted Average Farm Price per Bushel (USDA 2008)

average farm price (Figure 1.2) has increased steadily, most noticeably increasing 170% between 2006 and 2008 (USDA 2008).

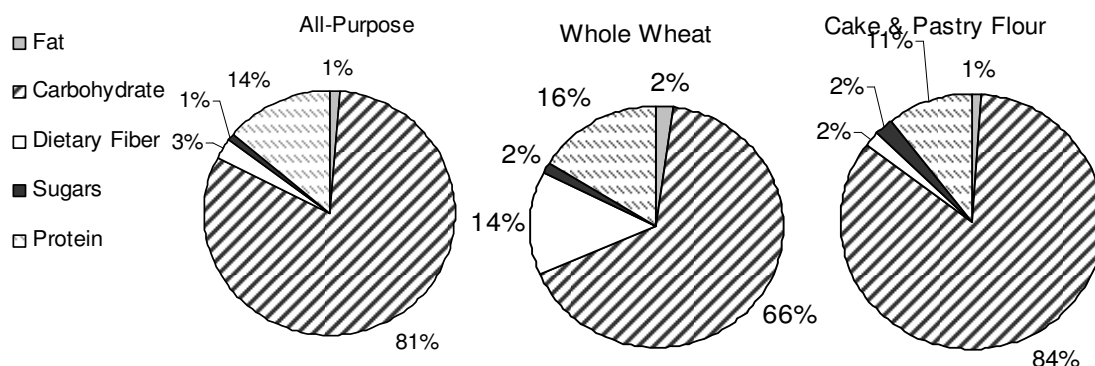
#### Wheat Fractions

Wheat kernels or berries are often milled into three fractions: the germ, embryo, and bran. The wheat endosperm is comprised primarily of starch with some protein and accounts for about 82% of the kernel weight (Ardnt & Luther 2008). The endosperm functions as a source of energy used in the germination and early growth of a new plant (Fulcher & Duke 2002). The bran is comprised of several cell layers and accounts for about 15% of the kernel weight. The bran is also primarily carbohydrate with a larger fraction (40%) of fiber as well as protein, ash and lipid. Germ is comprised of carbohydrate with fiber (13%), protein, the highest level of lipid in wheat, and ash. In addition, several vitamins, minerals, and other phytonutrients, are found in the germ. (Ardnt & Luther 2008)

#### Wheat Flours

##### **Flours**

Protein content of different flours is generally the most important consideration in baking and flour selection. Flours of variable protein content are available for specific baking purposes (Figure 1.3). Higher protein content is preferred when stronger dough is needed, that is, when dough needs to rise and hold a structure. Rising for yeast products occurs when yeast metabolizes sugars and produces carbon dioxide. When enough gluten is produced the dough will stretch and



**Figure 1.3: Macronutrient Comparison of King Arthur Commercially Available Flours (King Arthur 2008)**

hold the carbon dioxide gases with-in the dough causing it to inflate. Common pan bread needs approximately 11.5% protein in order to rise. The wheat grain contains approximately 12% proteins; however flour producers can use cultivars characterized by high gluten yields, and milling and drying techniques to vary the end protein content and vital gluten levels in flour (Borghet et al. 2005).

Improved wheat cultivar characterization and milling technologies have brought about specialty pastry and pasta flours. Whole wheat pastry flour is produced from the soft varieties with lower protein content than hard varieties and is a better selection for flaky pastries. Whole durum wheat is produced under the same requirements as whole wheat flour but use durum and red durum wheat (21 CFR Section 137.225). Durum wheat is generally used for pasta and noodles due to its high protein content. In all flours the percent moisture is altered during production of whole wheat flour through a process known as tempering so that the final moisture content does not exceed 15% (21 CFR Section 137.200).

Different classes of wheat are used to produce commercially available whole wheat, white whole wheat, and whole wheat pastry flours. Five of the six wheat classes: Hard Red Wheat, Hard Red Spring, Soft Red Winter, Hard White, and Soft Wheat may all be used to produce whole wheat flour (21 CFR. Section 137.200). White whole wheat flour is produced from white varieties of wheat that do not have the genes to produce the typical brown/red bran color. Whole wheat bread baked with white varieties also score higher among panelists in terms of flavor and mouth-feel (Taylor et al. 2005). White and red varieties are considered nutritionally equivalent. The Whole Grains Council states that white varieties have fewer phenolic compounds that give the wheat a milder flavor (WGC 2008). In contrast multiple studies indicate that while there are strain, environmental and storage impacts on antioxidant activity no studies to date have found a trend to indicate that white or red varieties are significantly different (Yu et al. 2002a, Yu & Zhou 2004a, Zhou & Yu 2004b).

### **Whole Wheat Flour**

Whole wheat flour, also known as graham flour, is flour produced in proportion to the original natural composition of the wheat kernel. Wheat kernels are composed of three fractions: bran, germ, and endosperm. Refined flour consists of only the endosperm with the bran and germ removed. The requirements for whole wheat and refined flours are set by the Food & Drug Administration, Department of Health and Human Services in The Code of Federal Regulations, Sections 137.200 and 136.105 respectively (21 CFR).

These requirements use particle size and ash to standardized flours. Removal of the bran is possible as it is less easily reduced in size and therefore may be sifted out. For whole wheat flour, cleaned wheat is ground so that 90% passes through a 2.36 mm sieve and 50% will pass through an 850  $\mu\text{m}$  sieve (FDA 2007). Ash content, the nonvolatile inorganic compounds, generally considered the mineral content in wheat, is determined by an AACC (Analytical Association of Cereal Chemists) method in which samples are incinerated overnight in a muffle furnace at 600°C. (FDA, 2007) Whole wheat flours have larger particles sizes and higher ash contents due to the presence of the bran fraction.

### *Health Promoting Properties of Whole Grains*

#### Epidemiological Studies

Epidemiological studies provide support that whole foods, such as those prepared with whole grains, have a greater effect against a broad range of diseases than isolated components (Slavin 2003). A meta-analysis of 12 studies demonstrated that regular intake of whole grain foods was capable of a 26% reduction in risk for coronary heart disease (CHD), possibly because of their ability to decrease serum LDL- cholesterol and triglycerides (Anderson et al. 2000). Women in the Iowa Women's Health Study and Harvard Women's Health Study with the highest consumption of whole grains (approximately three daily serving) experienced 22 & 27 % reduced risk of type II diabetes, respectively (Jones et al. 2002). Similarly, meta-analysis of 45 well designed studies found whole grains to be protective against cancer in 43 of the studies (Slavin 2000). Interestingly, while at least one report by

Arts and Hollman has challenged the role of antioxidants for their protective role against disease, no studies to date have reported an adverse effect to the consumption of whole grain with the exception of persons with disease complications such as celiac disease (Arts & Hollman 2005).

#### Dietary Fiber

Dietary fiber is associated with decreased risk of cardiovascular disease, diabetes, and intestinal cancer in response to reducing plasma and LDL-cholesterol, glycemic and insulin response delay, and increasing stool bulk (Schneeman 1999). Epidemiological studies have demonstrated that dietary fibers play a pivotal role in modulating food intake, body weight, glucose homeostasis, plasma lipid profile, and cardiovascular disease (Koh-Banerjee & Rimm 2003). Independent association of higher fiber intake with lower BMI or reduced food intake has been established (Kimm 1995, Pasman & Saria 1997, Burton & Freeman 2000, Cani et al. 2006, Melanson et al. 2006). Fiber may play a unique role in weight management for women. Women who consumed higher fiber diets were strongly correlated with lower BMI and that among these women; dietary fat intake had no relationship with BMI unless coupled with a high-fiber diet. Furthermore, different types of fiber and fiber in different forms may differ in their effectiveness to modulate weight loss. (Howarth et al. 2005)

Fiber supplementation studies on weight control indicate that fiber types may differ in their effectiveness. Soluble guar gum fiber was found to decrease energy intake by free living obese women by 19% over a one week period (Pasman & Saris



1997). In contrast, in one study neither fermentable or non-fermentable fibers had an effect on energy intake nor body fatness with an increase of 28 grams fiber per-day by fiber supplementation with fiber isolates over a three week period. In both fiber supplementation studies and bread formulas, authors suggest that the use of the fiber supplement vs the fiber of intact plants and the cellular structures associated with such dietary fiber in foods may have resulted in the unexpected results. (Holt et al. 2001; Howarth et al. 2003) Similarly, a separate study on the effects of dairy products and fiber on weight loss in obese adults found that subjects who consumed more high-fiber foods was greater than the weight loss of subjects who consumed fiber supplements (Thompson et al. 2005). When at least 34 grams of fiber per day were consumed, a 3-6% decrease in energy absorption was observed. To be effective, the fiber may need to be an integral part of food rather than in supplemental form. (Howarth et al. 2005)

Fiber rich foods may require more chewing, take longer to eat, and larger portion size of high fiber vs. high fat foods may induce satiety sooner. Reduced palatability and increased chewing effort and time may alter cephalic and gastric responses leading to satiety hormones reaching the hypothalamus earlier and reducing food intake. (Burton-Freeman 2000, Holt et al. 2001) Soluble fibers may elicit their strongest effect in bulking properties. MRI images demonstrate that alginates form lumps in the stomach which created a significantly greater sense of fullness when compared with a control (Hoad et al. 2004). While bulking properties are often cited as the primary fiber mechanism for satiety, highly insoluble wheat bran based breakfast cereal induced greater satiety than a high solubility psyllium gum breakfast

cereal (Delargy et al. 1997). Satiety from rice-based, wheat-based and rice-pulse combinations were higher for fermented and whole fraction based preparations than a white bread standard (Pai et al. 2005). Weight loss from fiber-rich whole-grain cereals was also shown to maintain or increase the intake of total and insoluble fiber, vitamin B-6, and magnesium, indicating that hypocaloric diets may be used as an effective means of weight loss without causing incomplete nutrition (Kimm 1995, Melanson et al. 2006).

High fiber foods may also regulate glycemic index and alter satiety hormone levels. Insulin sensitivity has been shown to decrease in whole grain diets compared with medium glycemic load compared with high glycemic load refined grain diets (Periera & Liu 2003). In healthy men the insoluble fiber content in high-fiber cereal reduced appetite, food intake, and improved glycemic response to meal consumption consumed 75 minutes post treatment (Samra & Anderson 2007). Low glycemic index diets have also been correlated with satiety with increased levels of cholecystokinin (CCK) independent of the glycemic response (Koh-Banerjee & Rimm 2003, Swinburn et al. 2004). Elevated plasma CCK levels patterns have been recorded in both low fat, high fiber diets as well as high fat, low fiber diets. Proximal fermentation of viscous fibers in the distal colon produce short chain fatty acids, an effect possibly caused by a delay in fat absorption (Burton-Freeman 2000; Cani et al. 2006). Likewise, ghrelin levels are decreased after carob fiber consumption possibly related to alterations in lipid metabolism (Gruendel et al 2007). Similarly ghrelin and PYY levels were blunted after wheat fiber yet levels were not blunted for oat-fiber, however hunger scores were not significantly different (Weickert et al. 2006).

## Antioxidant Activity & Energy Balance

Correlation between antioxidant activity and energy balance has recently, albeit in limited amount, been studied. Antioxidants are thought to alter satiety effects by; lowering blood glucose response, reducing lipid oxidation, impeding glucose uptake by cells, and modulation of digestive enzymes. Specifically, wheat bran, known to contain antioxidants, improves glucose tolerance over pectin administration, possibly by regulation of oxidative stress. Vitamin E plays a role in insulin metabolism and the prevention or alleviation of hyperinsulinemia. (Koh-Banerjee & Rimm, 2003) Administration of green tea polyphenols in diabetic (db/db) mice results in increased levels of yet unidentified pancreatic polypeptides previously found to reduce food intake and increase energy expenditure (Tsuneki et al 2004). In contrast, body weight was restored in alloxan-induced diabetic Sprague-Dawley rats when treated with feruloyl oligosaccharides. The in vivo antioxidant restoration capacity is cited for mitigating the oxidative damage responsible for the degeneration of pancreatic  $\beta$ -islet cells, exerting hypoglycemic activity by stimulating insulin release. (Ou et al 2007) This is a key finding in the treatment of hyperglycemia and the prevention of atherosclerosis in addition to energy balance.

Polyphenols may increase lipid oxidation and energy expenditure in humans and mice. Lipid oxidation was significantly increased for a liquid meal challenge when a high fiber polyphenol rich Carob fiber supplement was given in human subjects. Interestingly, acylated levels of ghrelin, a meal inducing hormone, were decreased indicating that carob fiber may influence the regulation of energy intake

and body weight. (Koebnick et al. 2006, Gruendel et al. 2006) In contrast, a separate study on Wistar rats found that hypothalamic reactive oxygen specie production was required to elicit a satiating effect during hypertriglyceridemia. Food intake during hypertriglyceridemia is reduced by GSH-EE, a potent ROS scavenger, and trolox, a water soluble vitamin E analog, indicating that ROS-sensitive mechanisms may be sufficient to promote satiety. In metabolic diseases such as obesity and diabetes, ROS may be chronically elevated and therefore hypothalamic sensing of fat through ROS targets for hypertriglyceridemia-induced satiety may be a potential redox-regulated system useful for regulating energy homeostasis. (Benani et al. 2007)

Polyphenolics may reduce energy intake by impeding glucose intake into cells. Green tea polyphenolics such as Epicatechin gallate inhibit the transportation of glucose by binding to the glucose transporter in an antagonist like manner (Kobayashi et al. 2000). Similarly, isolated forms of (-)-epigallocatechingallate, (-)-epicatechingallate, and epigallocatechin were found to be effective against both sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) in Caco-2 cells (Johnston et al. 2005). A separate study utilizing New Zealand black mice found that epigallocatechin gallate (EGCG) attenuated diet-induced obesity. While the mechanism was not clear, it was hypothesized that higher fecal energy levels in EGCG treated mice indicates reduced diet digestibility, while there may have also been a metabolic effect on the liver and white fat. (Klaus et al. 2005) Glucose transporters 1 and 4 may be inhibited by genistein, quercetin, myricetin, morin, rhamnetin, and isorhamnetin, and flavonoids, including catechin, respectively. Thus, at concentrations of 10-100 $\mu$ M quercetin, myricetin, and catechin-gallate dose

dependently inhibited the uptake of methyl-glucose in rat adipocytes. (Strobel et al. 2005)

Intestinal activity of polyphenols against starch degradation may also reduce energy intake. Plant polyphenolic extracts have demonstrated comparable effectiveness against  $\alpha$ -glucosidase and maltase activity as acarbose and voglibose, two synthetic inhibitors used to control non-insulin dependent diabetes mellitus. While *in vitro* activity is limited due to bioavailability, diacylated anthocyanins are particularly effective against  $\alpha$ -glucosidase, most likely due to competitive binding although the structure-activity relationship has not been fully explained. The binding of digestive enzymes, particularly  $\alpha$ -amylase by polyphenolic-rich plant extracts may explain in part the insulin-like action of polyphenolics. (McDougall & Stewart 2005) Thus, polyphenolics through a variety of mechanism are potential natural alternative to assist in energy balance through diet-mediated means.

#### Government Approved Health Claims

The nutritional value of wheat and other whole grains has lead to multiple whole grain food intake recommendations. The 2005 Dietary Guidelines for Americans report recommends increased consumption of whole grains as part of a health-promoting diet, stating a diet rich in whole grain foods may reduce the risk of coronary heart disease, type 2 diabetes and certain cancers and help with weight management (Marquart et al. 2003). It recommends that half of daily grain intake should be from whole grains, which equates to three ounce-equivalent servings daily for a 2,000 calorie diet (USDA 2005). Increased consumption of whole-grain foods

is being campaigned for in the UK with “Whole Grains for Health” and in Australia with “Go Grains”. Unfortunately, whole grain consumption remains low with less than 10% of American adults meeting the recommended intake (Cleveland et al. 2000). Whole grain food consumption may increase with consumer education and allowed health claims such as the FDA approved the health claim for whole-grain products containing at least 51% whole grain by product weight and meeting other criteria in the U.S (FDA 1999).

#### Whole Wheat Product Market

The whole-grain market in the United States is on the rise with inclusion of whole wheat in products such as crackers and breads (Carlson et al. 2005). In addition to the FDA approved health claim, merchants creating whole-grain foods may also signify their product with the whole grain stamp. The stamp was launched by the Whole Grains Council, a group of millers, manufacturers, scientists, and chefs have advocated for research on whole grains as well as their use and identification in the marketplace. Additional sources report a 168% increase from 2004 to 2005 in frozen whole grain prepared foods, followed by whole grain pasta, whole grain cereal, and whole grain bread and baked foods. (The Whole Grain Council 2007) A study of British adults found current whole grain consumption levels similar to those in the United States. Of the small percentage of whole grains consumed 40% came from breads, and about one-third from whole grain breakfast cereals (Lang et al. 2002). The remaining portion came from whole grain pasta, rice, and other cereals.

Development of more desirable whole wheat products and recipes may increase consumer attitudes toward these products.

### Functional Foods

#### Functional Foods and Nutraceuticals

Functional foods are defined by the International Food Information Council (IFIC 2007) as, “foods or dietary components that may provide a health benefit beyond basic nutrition.” Nutraceuticals is a term often used synonymously with functional foods or as a broader term that incorporates functional foods as well as the functional ingredient in a supplemental form. In Japan functional foods have been regulated for over 15 years as Foods for Specialized Health Use (FOSHU) (Warfel et al. 2007). Regardless of the title, functional foods are generally defined as foods which contain biologically active components (often termed bioactive compounds) which may exert beneficial physiological health effects and reduce the risk of chronic disease. As research to identify beneficial health components of foods increases, many traditional foods may be labeled or modified to become functional foods.

#### Functional Food Outlook

Functional foods and beverages have a bright outlook as the largest single domestic market in the US with an estimated \$29 billion in 2007 sales according to Global Industry Analysts (Douaud 2007). The market in Japan, the only nation to legally define functional foods, has grown from \$1 billion in 1997 to \$6 billion in

2006 (Warfel et al. 2007). Canada has shown a similar trend with health promoting products comprising six out of seven food and beverage product categories with the greatest growth in sales rates (Cranfield et al. 2006). Thus, the market demand has created new opportunities in the agricultural and food sectors and encouraged new innovations, while potentially promoting the well-being of its consumer base.

According to Mintel Global New Products Database (Mintel 2007), launches of new food products with a "whole grain" claim have grown considerably since 2000. Launches of new whole grain products increased over 900% from 2000 to 2006 reaching nearly 1,400 new products (Mintel 2007). Bakery products, breakfast cereals, snacks, and side dishes accounted for most new products (**Table 1.1**). Despite increased availability of foods made with whole grains and strong recommendations to increase whole grain consumption, Americans currently consume an average of one daily serving of whole grains, which is one-third of the recommended intake.



**Table 1.1** New product introductions with a “whole grain” claim.

<b>Category</b>	<b>2000</b>	<b>2001</b>	<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>
Baby Food	3	3	6	1	8	0	20
Bakery	80	109	149	205	309	291	570
Beverages	0	0	0	1	2	2	3
Breakfast	31	80	68	98	138	192	347
Cereals							
Confectionery	3	2	1	1	2	1	0
Dairy	3	4	4	5	3	3	10
Dessert & Ice Cream	0	0	0	0	0	2	2
Fruit & Vegetables	0	0	0	0	2	0	1
Meals & Meal Centers	7	3	12	9	25	20	65
Pet Food	1	1	0	0	6	7	14
Processed Fish, Meat, Egg	7	1	0	2	8	2	2
Sauces & Seasonings	0	1	1	1	1	6	9
Side Dishes	16	18	45	33	44	93	117
Snacks	3	22	15	30	58	83	227
Soup	0	0	1	0	3	1	0
Spreads	0	0	0	1	0	1	0
<b>Total</b>	<b>154</b>	<b>244</b>	<b>302</b>	<b>386</b>	<b>609</b>	<b>704</b>	<b>1387</b>

\* Table taken from Arndt and Luther 2008

Less than 10% of adult Americans meet the recommended whole grain consumption level (Cleveland et al. 2000). The 2010 goal of the ‘Healthy People Goals and Objectives for the Nation’ by the U.S. Department of Health and Human Services is that at least 50% of Americans would consume three whole-grain servings daily.

#### Nutraceutical Components

Compounds found in food products, such as carotenoids, phenolics, fatty acids, dietary fiber, plant sterols, and pre-/pro-biotics, support health. Bioactive

compounds, such as carotenoids and phenolics, protect health through antioxidant activity against highly reactive free radicals and oxygen species. Protection of body proteins, lipids, and DNA can play a vital role in preventing degenerative diseases such as heart disease, cancer, cataracts, brain dysfunction, and arthritis (Miller et al. 2000). Essential fatty acids (EFA) are fatty acids which are required from the diet and play an important role in growth, skin health and neurological function (IFIC 2007). There is a lot of potential for new reduced cost EFA functional foods containing fatty acids such as omega-3 long chain polyunsaturated fatty acids, as EFA's are found in high-value foods such as fish and walnuts. Such bioactive compounds play a key role in the production and protection of body cells and their components.

Plant sterols and pre/pro-biotics play a major role in the gastrointestinal tract. Plant sterols are thought to lower serum cholesterol as their structure closely resembles cholesterol inhibiting its absorption while not being readily absorbed themselves (Jenkins et al. 1999). A final example is the use of pre- and pro-biotics for healthy gut function. Probiotics are viable microbial strains that beneficially affect the host, and prebiotics are non-digestible food components that selectively stimulate the growth and activity of selective bacteria. Intake of pre- and pro-biotics may boost the immune system, reduce rotavirus-induced diarrhea, alleviate symptoms of lactose intolerance, and reduce the risk of colon cancer. These examples are a few of what is currently known about the effects of different types of bioactive components and their effect on human health.

## Antioxidants

### Antioxidants and Human Health

Correlation between oxidative stress and reactive oxygen species (ROS) or reactive nitrogen species (RNS) with chronic aging or disease states has increased interest in the role of dietary antioxidants to prevent these conditions and promote health. Many definitions for antioxidants can be found. The Halliwell and Gutteridge broad definition for antioxidants is “any substance that when presented at low concentrations, compared to those of an oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate” and has remained a prominent definition used in the sciences (Gutteridge & Halliwell 1999). Of growing familiarity is the Institute of Medicine proposed definition, “A dietary antioxidant is a substance that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans” (Food and Nutrition Board 1998). Antioxidant research is important in the field of food science for both their potential to stabilize food systems and their potential health benefits.

Perhaps the strongest evidence for the role of antioxidants in human health are the epidemiological studies indicating that a diet rich in foods containing antioxidants such as fruits, vegetables, and other plant derived foods decreases the risk of certain chronic diseases (Wattanapitayakul & Bauer 2001, Stanner et al. 2003, Wilcox et al. 2008). A comprehensive review of the intervention trials containing more than 1,000 subjects studying the correlation between antioxidants and cardiovascular disease concluded that, “people with a high occurrence of CVD often have low intakes or plasma levels of antioxidant nutrient” (Stanner et al. 2003). The Nurses’ Health

Study and the Health Professionals' Follow-up Study found a 4% reduction in CHD for each 1-serving/day increase in the intake of fruits and vegetables when comparing the highest ( $\geq 8.0$  servings/day) and lowest ( $\leq 3.0$  servings/day) quintiles (Kris-Etherton et al. 2002). Supplementation with single or combinations of antioxidant have not been found to prevent CVD (Kris-Etherton et al. 2002).

Vitamin E intervention studies are divided with the Nurse's Health Study supplementing for  $\geq 2$  years and at specific doses reducing the relative risk to 0.66, while the more recent Physicians Health Study found no significant impact of Vitamin E and CVD relative risk, and a MEDLINE meta-analysis does not support the routine use of vitamin E for the prevention of CVD (Rimm et al. 1996, Vivekananthan et al. 2003, Wilcox et al. 2008). The difference between epidemiological study and intervention study observations may be due to the differences in the populations studied, or that the follow-up time required to identify clinical benefits of Vitamin E supplementation may be decades long, as antioxidants are thought to be most effective at combating ROS-induced atherosclerotic lesion formation (Wattanapitayakul et al. 2001, Steinhubl 2008). Vitamin E serves as one example of the multiple bioactive components and intake conditions that may affect their effect on health that have been studied (Fang et al. 2002, Kris-Etherton et al. 2002, Padayatty et al. 2003, Polidori 2003, Frei 2004). Due to the expensive nature of intervention studies, the quest to understand the mechanism of action of antioxidants have lead to *in vitro* and *in vivo* model development to quantify foods with antioxidant activity (Nandakumar et al. 2008).

## Methods of Antioxidant Analysis

Oxidative defense mechanisms, protection from free radicals, is a primary mechanisms investigated to combat aging and age-related diseases (Wilcox et al. 2008). Free radicals are molecules with an unpaired electron in the outer orbital shells, creating generally unstable and highly reactive conditions (Fang et al. 2002). Therefore, it is possible to measure antioxidant activity of foods, and food components by exposing them to reactive oxygen species. Mechanisms by which antioxidants are postulated to scavenge these free radicals include hydrogen atom transfer (HAT), single electron transfer (ET or SET), and sequential proton loss electron transfer (SPLET) (Moore & Yu 2008). Detailed descriptions of these radical scavenging capacity assays are available in Shahidi & Hos' book Antioxidant Measurement and Applications and more specifically Moore & Yu's chapter Methods for Antioxidant Capacity Estimation of Wheat and Wheat-Based Food Products, in Wheat Antioxidants (Shadihi & Ho 2005, Moore & Yu 2008).

### Wheat Antioxidants

#### Antioxidants in Wheat Fractions

Phytonutrients including phenolic acids, flavonoids, coumarins, polyphenols, phytates, terpenes, carotenoids, tocopherols, and tocotrienols are all antioxidant compounds found in wheat (Shahidi 2004). These antioxidant compounds may act to improve health by scavenging free radicals, chelating transition metals, quenching singlet-oxygen molecules, acting as a reducing agent, and defense enzyme system activators for biological systems (Moore et al. 2005, Leenhardt et al. 2006).

Antioxidants are concentrated in the outer parts of the kernel, particularly the endosperm aleurone layer, pericarp, nucellar envelope and germ. Some bioactives such as zeaxanthin have been reported at higher levels in the germ fraction while some components such as carotenoids and lutein are distributed throughout the whole grain (Leenhardt et al. 2006). When compared with the endosperm fraction, combined bran and germ fractions contain 15-18 times more total phenolics, 52-70 times more ferulic acid, and on average 4 times more lutein, 12 times more zeaxanthin, and 2 times more cryptoxanthin (Zhou et al. 2004b, Adom et al. 2005, Moore et al. 2005). The total antioxidant capacity (TAC) as measured by  $\mu$ mole Trolox equivalents/100 g wheat portion, found that wheat germ had the highest TAC value with an average of 8,400 followed by wheat bran and refined wheat flour fractions at 3,500 and 1,450  $\mu$ mole Trolox equivalents/100 g, respectively (Arndt & Luther 2008).

#### Effect of Storage & Processing on Wheat Antioxidants

A multitude of factors may impact the final antioxidant activity in wheat samples and final wheat based foods. Environmental factors including temperature, solar radiation and growing location have been shown to significantly impact antioxidant properties (Yu et al. 2003, Zhou et al. 2004a, Zhou et al. 2004c). Genotype has also been found to significantly impact antioxidant levels in some wheat varieties. Chinese black-grained wheat has higher antioxidant activity than white and blue varieties (Li et al. 2007). A study of Maryland-grown soft wheat also found significant differences between varieties suggesting that it may be possible to

produce soft wheat varieties with higher antioxidant activity through cultivation (Moore et al. 2005).

Post harvest activities may also significantly impact final antioxidant activity in wheat foods. Cheng et al. reported on the effects of mesh size on the availability of wheat antioxidants indicated that storing whole grains as opposed to smaller fractions is preferable for maintaining natural antioxidants (Cheng et al. 2006). To date, multiple studies confirm that wheat antioxidants are asymmetrically distributed throughout the wheat kernel (Zhou et al. 2004a, Gallardo et al. 2006, Liyana-Pathirana & Shahidi 2006, Liyana-Pathirana & Shahidi 2007). A significant portion of wheat's antioxidants are located in the bran fraction (Zhou et al. 2004b, Liyana-Pathirana & Shahidi 2007) Zhou and others studied milling size and concluded microionization of the aleurone fraction improved the availability of the wheat antioxidants (Cheng et al. 2006).

#### Antioxidants in Wheat Based Food Products

Losses of vitamins and antioxidants have been reported in breadmaking and production of other wheat based foods. A study on French and wheat breadmaking found that there were significant losses in tocopherols and tocotrienols during the dough formation and baking stages (Wennermark et al. 1992). Oxygenation during dough production and heat destruction during baking are speculated causes for 30% decreases in Vitamin E content during bread preparation (Leenhardt et al. 2006). Carotenoid losses in the kneading stage varied by species and were directly correlated with lipoxygenase activity (Leenhardt et al. 2006) .

In bread wheat, the carotenoid content decreased 66% in whole and 40% in refined grain dough after kneading. By comparison, there was less than 10% loss in carotenoids in whole grain einkorn dough after kneading. Einkorn has a much higher carotenoid content and lower lipoxygenase activity compared to bread wheat. Carotenoid losses during fermentation and baking were about 10% and 36-45% respectively and did not differ appreciably between wheat species. (Leenhardt et al. 2006) These losses are higher carotenoid losses of approximately 20% for bagels and cakes, 30% for cookies, and total carotene losses of 4-15% in bread products and 18-23% in crackers in other baking studies (Ranhotra et al. 1995, Park et al. 1997).

Fermentation processes, such as those used in sourdough bread production, may impact antioxidant levels and bioavailability. Katina and others found that extracts of flour that have undergone sourdough fermentation have increased folate and total phenolic content compared with unfermented flour (Katina et al. 2005). Alternatively, alkylresorcinols (ARs) were diminished in sourdough wheat bread as opposed to wheat flour ; however extraction of ARs may not have been complete due to AR-starch complexed within the food matrix (Ross et al. 2004). In a whole wheat bread system, sourdough fermentation resulted in greater reduction in phytate (62%) compared to yeast fermentation (38%), while increasing the solubility of magnesium and phosphorus (Lopez et al. 2001). Thus, phytonutrient composition of dough's may vary at different stages of bread formation and in the final product.

Baking conditions may also alter antioxidant content. Increasing baking time and temperature increases antioxidant activity in the bread crust, and lowers antioxidant activity in the bread crumb portion (Lindenmeier et al. 2004). Toasting



bread has also been found to increase antioxidant content 134-367% in bagels, French bread, white bread and whole wheat bread compared to their un-toasted counterparts (Halvorsen et al. 2006). Maillard reaction products have received significant attention as they may produce potential carcinogens; however, study of bakery product fractions also indicate that areas subject to the browning reaction have higher antioxidative potential in contrast to their corresponding inner counterparts (Lindenmeier et al. 2004).

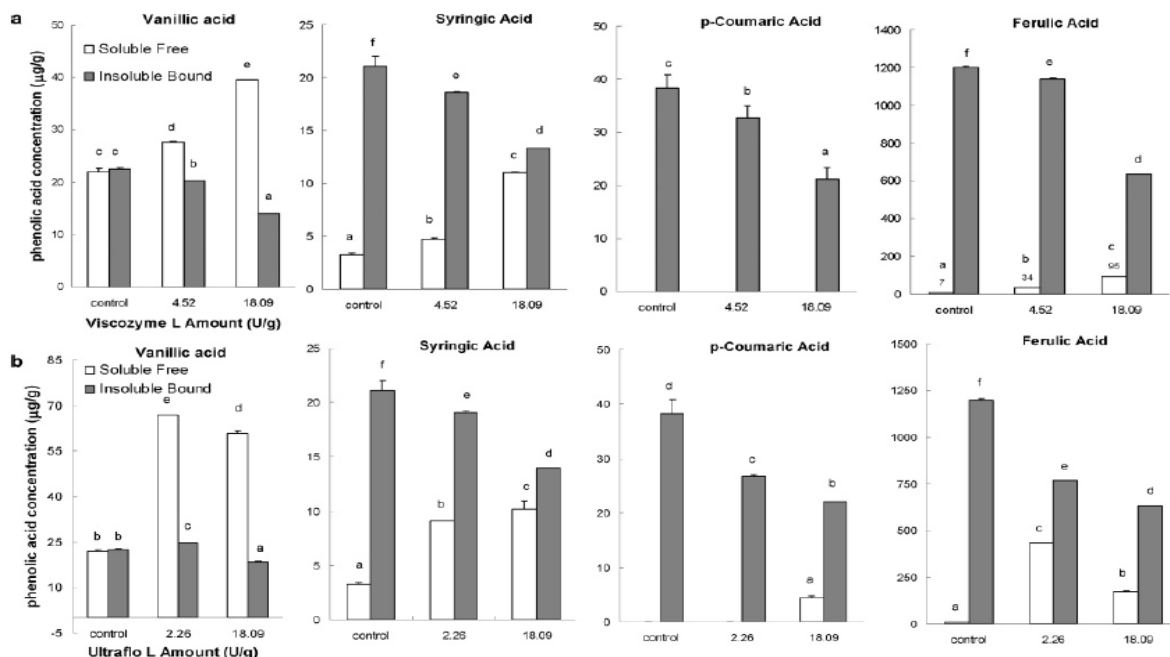
The most important factor in determining the antioxidant capacity of wheat products is the use of whole wheat rather than refined wheat flour. Halvorsen and others found that the antioxidant content of whole wheat bread is more than 1.9 times higher compared to refined wheat bread (Halvorsen et al. 2006). Unfortunately, straight bran supplementation was found to weaken the dough structure resulting in smaller bread loaves with poorer crumb quality (Salmenkallio-Marttila, et al. 2001). Miller et al. found the same trend with higher antioxidant content in whole grain cereals and breads compared to their refined grain equivalents (Miller et al. 2000). Similarly, the average antioxidant content of ready to eat (RTE) breakfast cereals was found to be higher than selected fruits and vegetables with the exception of berries, and whole grain cereal containing raisins was only slightly lower than berries. Antioxidant activity measured in  $\mu$ moles Trolox equivalents/100 g dry cereal ranged from 1,300 to 3,900 among 17 different refined, whole grain and bran-based RTE cereals, with bran-based RTE cereals higher than that of whole wheat cereals. (Miller et al. 2000). Baublis and others studied the antioxidant effects of wheat bran-, whole grain wheat- and refined wheat-based RTE cereals using aqueous extracts prepared from

crushed cereal samples. Both whole wheat and wheat bran-based RTE breakfast cereals were shown to inhibit lipid oxidation promoted by iron and peroxy radicals while the refined wheat RTE cereal aqueous extract showed little antioxidant capacity. (Baublis et al. 2000)

### *Effect of Solid State Reactions on Antioxidants*

#### Solid State Reactions

Solid state fermentation utilizes food grades bacteria or yeast at a low moisture content to release or produce beneficial components. Phenolic acids, major contributors to the antioxidant properties in wheat, principally exist insolubly bound to cell wall material in the bran fraction. Phenolic acids in the bound form are not readily bioavailable, with only 3% of ingested bran phenolics ingested over a 24 hour period in humans. (Moore et al. 2007) Multiple studies evaluating wheat variety, growing conditions, post harvest treatment, particle size, temperature, enzyme treatment, and yeast treatment demonstrate a capacity to affect antioxidant activity and potential bioavailability of antioxidants (Zhou et al. 2004a, Yu & Zhou 2004, Moore et al. 2006). As shown in **Figure 1.4**, enzyme treatment of wheat bran was able to convert vanillic acid, syringic acid, and up to 50% of the existing ferulic acid, the primary phenolic acid in wheat, from insoluble bound to the soluble bound form (Moore et al. 2006b). Barley and wheat malt treated with brewers yeast also demonstrated a release of ferulic acid during fermentation through enzymatic activity (Coghe et al. 2004).



**Fig. 1.4** Effects of enzyme treatments (a) Vicozyme L and (b) Ultraflow L on the phenolic composition of Akron Wheat bran. Results are expressed as micrograms of individual phenolic acids per gram of wheat bran on a dry weight basis. The mean values of duplicate tests are reported. Vertical bars represent the standard deviation and values marked by the same letter are not significantly different ( $P < 0.05$ ). (Moore et al 2006)

## Nutritional Outreach

### Outreach Development Tools

Effective development, distribution, and analysis of nutritional outreach programs is a complex project requiring the knowledge and skills from nutritional scientists, dieticians, public health specialists, informatics specialists, and educators among others. Therefore, this segment will not attempt to cover all of the considerations for these fields, but rather to give a complete overview of the essential components used in the development of a specific food science, nutraceutical based outreach program. These essential components are survey methodology, message framing, literacy and language, and technology.

## Outreach Materials

The science of communication has recently been tapped to assist in using technology for nutrition outreach. Tailored messages that speak personally to targeted audiences have a significantly greater chance of making an impact on health (Wilson 2007). Unfortunately, funding for public health campaigns pales in comparison with food industry advertising dollars and therefore consumers have more exposure to nutritionally dubious food products than health information (Johnson-Taylor et al. 2007). New technologies such as telenutrition, the use of interactive compressed video technology to deliver medical nutrition therapy, may also prove fruitful. Telenutrition allows urban dietitians to counsel patients' long distance. Thereby, specialists can reach patients in remote areas, eliminating travel, and extra financial and time expenditure. (McCabe et al. 2001)

Language and culture are also key factors important for effective nutrition outreach. Hispanics are the single largest ethnic group in the United States and Hispanic children are three times more likely to be overweight when compared with white companions (Mennella et al. 2006). It is well accepted that many factors influence food selection. Significant differences in early flavor and food experiences exist between Hispanic children when compared to non-Hispanic children. Dietician awareness may assist in the development of evidence based strategies to improve Hispanic children's eating habits and lower their risk of becoming overweight and developing other chronic diseases. (Mennella et al. 2006) In addition, review of Spanish-language versions of the U.S. household food security survey module found a *“lack of semantic equivalence across languages; lack of conceptual equivalence*

*across cultures; and lack of normative equivalence across societies.”* Thus, direct translation of materials may not be as effective as materials tailored for culture as well as language (Harrison et al. 2003).

#### U.S. Food Assistance Programs: Education & Nutrition

Domestic food assistance programs are essential for promoting health and fighting hunger in the United States. In the 2008 Farm Bill, the U.S. Congress allocated an additional \$10.361 billion for nutrition programs to combat rising food prices and growing food insecurity (House Committee on Agriculture 2008). The bulk of this funding is distributed through three programs: the Supplemental Nutrition Assistance Program (SNAP) formerly the Food Stamp Program, The Emergency Food Assistance Program (TEFAP), and the Fresh Fruit and Vegetable Program under the Richard B. Russell National School Lunch Act (Rosenbaum 2008). These programs and their subsidiaries are challenged with the twofold task of feeding America’s hungry while sustaining or improving nutrition, specifically preventing further increases in obesity. For the purpose of this paper, a focused review of literatures on the SNAP program, and their evidence for cooking activity inclusion follows.

The Supplemental Nutrition Assistance Program (SNAP) is the largest of the federal food assistance and nutrition programs. This program serves the nations poorest who have a gross and net income below 130% and 100% of the poverty line, respectively (Landers 2007, Bergeson 2008). Advantages of SNAP are; eligibility is need based and all individuals meeting the financial guidelines can receive benefits, the thrifty meal plan outlines inexpensive, nutritionally adequate diets, and many

participants are working while receiving food stamps as the stamps are intended to supplement food purchases. Food stamps are intended to allow participants to obtain a low-cost nutritionally adequate diet.

The idea of restricting food purchases with food stamps has been debated since 1950 when the House version of the program bill would have prohibited the purchase of soft drinks and so-called luxury foods (Landers 2007). To date, with the exception of alcohol and imported foods, no restrictions exist. Of great concern is also what participants are not purchasing. SNAP participants were less likely than income-eligible nonparticipants to purchase fruits and vegetables, albeit most Americans fail to meet the recommendations for these foods (Guthrie et al. 2007).

The most compelling evidence for a change in the SNAP program is the health status of participants. Most studies indicate that SNAP participants have a higher risk of being overweight and obese as compared with low-income counterparts, particularly for females (Gibson 2003, Gibson 2006, Webb et al. 2008). Recently, when compared to the national average, both male and female SNAP participants have achieved equivalent weight status to the general population, mostly due to increase in obesity and overweight of nonparticipants (Guthrie et al. 2007). With 26 million low-income Americans participating in SNAP, policy and education changes to the program could yield significant benefits in reduced medical costs, lost wages, and increased productivity.

Shortcomings of SNAP to improve the nutritional status lead to the official food stamp nutrition education amendment of 1981. This amendment includes, “[to] encourage the purchase of nutritious foods, the Secretary is authorized to extend food

*and nutrition education to reach food stamp program participants, using methods and techniques developed in the expanded food and nutrition and other programs* (Landers 2007).” The Expanded Food and Nutrition Education Program (EFNEP) is an education program under the USDA. The mission of EFNEP is to: *“assist limited-resource audiences in acquiring the knowledge, skills, attitudes, and changed behavior necessary for nutritionally sound diets, and to contribute to their personal development and the improvement of the total family diet and nutritional well-being* (USDA-CREES 2008).” In 2002, the Food Stamp Nutrition Education (FSNE) National Report was published through the Cooperative Extension/Land-Grant University System. The development team identified four areas that would lead to increased access to nutrition education and nutritious foods (Little & Newman 2002). This report outlines the four focus areas for food stamp participants: quality/physical activity, food security, shopping behavior/food resource management, and food safety (Guthrie et al. 2006).

The USDA Thrifty Food Plan (TFP) not only provides a basis for Food Stamp allotments, but an economic food plan for good shopping behavior & food resource management. The TFP has 15 “market baskets”, essentially shopping lists which meet the Dietary Guidelines for Americans and cost no more than the Food Stamp allotments. These market baskets are largely based upon raw food items which lower costs; however they increase food preparation time, kitchen equipment, and cooking knowledge. A limited study of SNAP participants found some participants lacked basic cooking skills and were unfamiliar with cooking from a recipe (Rose 2007). Americans, particularly young adults generally have low levels of food preparation

knowledge and are therefore unable to purchase and prepare foods for healthy meals (Michaud et al. 2007). SNAP participants, and others with limited income may be less likely to try new recipes or make suggested dietary changes as these changes would risk spending valuable resources on foods that their families may dislike, not eat, or recipes that may not turn out (Michaud et al. 2007). Hands on cooking activities have successfully increased cooking skills, confidence, and eating habits of preschool children and their families, college students, and WIC participants (Bensley et al. 2006, Condrasky et al. 2006, Dougherty & Silver 2007, Michaud et al. 2007).

Confounding the change from a provisional based program to an education program is the development of a process for validating outcome measures relevant to the target areas (Guthrie et al. 2006, Taylor-Powell 2006, Townsend 2006, Dollahite et al. 2008). Program evaluation, necessary under the Government Performance and Results Act of 1993, and emphasized in a 2004 U.S. Government Accounting Office report, while complex, may help pull the nutrition community together and highlight promising and proven programs (Guthrie et al. 2006, Taylor-Powell 2006). While education remains optional for both States and SNAP participants, similar nutrition outreach programs such as EFNEP with strong emphasis in education have recorded both health and expense benefits.

### Cooking Ability & Health

Increasing cooking knowledge, skills, and behavior may help improve healthy eating behaviors (Marquis et al. 2001, Levy & Auld 2004, Michaud et al. 2007). Estimates from the 1990's predicted that by the year 2000 the majority of U.S. adults



would have become dependent upon pre-prepared microwavable meals as to no longer be able to cook a meal for themselves (Beck 2007). The debate is further complicated by the use of commercial foods at different stages of preparation. For example, only 22% of weeknight meals prepared by middle-class parents in Los Angeles used little to no commercial foods; however, 53% demonstrated some independent cooking ability to prepare a meal not dominated by commercial foods (Beck 2007). The debate over the decline of cooking skills, cooking interventions, and commodity kitchens all give insight into the relationship of kitchen habits and health today.

Michaud et al. provide a thorough explanation of cooking skills and behaviors as a combination of mechanical cooking skills, perceptual skills, planning skills, and academic knowledge of food safety, nutrition, and culinary arts (Michaud et al. 2007). No studies have been reported that encompass all of these elements; however cooking skills and academic knowledge of nutrition and food safety have been researched. Cooking confidence and ability have been identified as key factors in obtaining healthy foods (Condrasky et al. 2006, Michaud et al. 2007). Young adults that purchased their own food and prepared meals at home had improved diet quality over their counterparts who did so less frequently (Larson et al 2006a, Larson et al 2006b). Among college students, 14% and 15% of male and female undergraduate students respectively self reported having little or no cooking experience and 23% and 18% of male and female 18-23 years old students, respectively, self reported having inadequate cooking skills (Hertzler & Bruce 2002, Larson et al. 2006a).

Pilot studies of cooking classes to improve the use of commodity foods for low-income families found that participants increased use of commodities and had a more positive attitude towards eating correctly (Auld & Fulton 1995, McLaughlin et al. 2003, Stead et al. 2004, Larson et al. 2006a, Larson et al. 2006b, Wrieden et al. 2007). Collective kitchens are a second approach to nutrition and food safety education that may also empower participants (Marquis et al. 2001, DeHaven et al. 2004, Engler-Stringer & Berenbaum 2005, Engler-Stringer & Berenbaum 2006, Wrieden et al. 2007). In addition to providing food to food insecure populations, collective kitchens have provided valuable insights into cooking-related needs such as cookware, literacy levels, language, perceived and actual cost barrier, and transportation among others (Kempson et al. 2003). Collective kitchens programs exist in different models; however, even non-funded health programs in faith based organizations have been shown to improve health and be more effective at reaching some at-risk populations (DeHaven et al. 2004).

Exploratory trials of collective kitchens emphasizing community-based food skills indicate a small but positive effect on food choice and confidence in food preparation. All such studies indicate the need for flexibility of such studies for reasons such as: poverty, food access, taste preference, literacy rates, and anxiety. Popular topics among participants in the UK were sauces, budget cooking, and soup. Younger respondents were more likely to be interested in less traditional dishes, yet overall vegetarian cooking along with vegetables, healthy cooking, and fish were the least popular topics. (Stead et al. 2004) Cooking intervention with adults in socially deprived areas of Scotland lead to a 12% decrease in convenience foods, an 18%

increase in ability to follow a recipe, an 8% increase in cooking from basic ingredients, a 27% increase in cooking lentil soup, and a 22% increase in cooking white sauce confidence in a 6-month follow-up compared with controls (Wrieden et al. 2007). All studies indicate that cooking intervention, particularly after multiple interventions, lead to increased nutrition, however word choice, recipe choice, and societal awareness are all important factors in determining the success of the intervention.

#### Impact of Nutrition Knowledge on Health

The American diet has become increasingly energy-rich and nutrient poor, a diet that the World Health Organization has linked with the world-obesity epidemic. The relationship between nutritional knowledge and eating behavior remains controversial. Multiple studies including one by the USDA found that improving nutrition knowledge has a small but measurable impact on improved diet quality among certain individuals. Other studies have not found a correlation between nutrition knowledge and diet selection or weight control indicating that knowledge alone may be insufficient to elicit diet related behavior change. (Frazao & Allshouse 2003, Klohe-Lehman et al. 2006) Outreach materials to improve dietary selection may require a combination of strategies and interventions, tailored to the needs of specific people groups (Frazao & Allshouse 2003).

Cost is a major factor influencing food purchases. However, short term economical purchasing of energy-dense diets high in refined grains, sugars, and fats may lead to diseases that are costly to treat, reduce longevity, diminish quality of life, and may be fatal. The highest rates of obesity and diabetes in the United States are found among minorities and working poor. (Drewnowski & Darmon 2005) Approximately 64% of American adults are considered obese. Direct costs for obese individuals in 2003 for medical expenditures were \$75 billion, 36% higher than their non-obese counterparts. When indirect costs such as loss of work, are included the severe economic impact that the obesity epidemic may incur is quite clear. (Seidell, 2003) Consumer education and the development of economical, health promoting foods is imperative to provide all economic groups with the opportunity to prevent the onset of diet related diseases.

Dollahite et al calculated that for every dollar spent under EFNEP there was a benefit-to-cost ratio ranging from \$9.58 - \$0.20 for direct and indirect benefits. Taking into consideration benefits such as future health care saving and avoiding or delaying loss of productivity the Virginia EFNEP program reported a \$10.63 per \$1.00 spent by avoiding or delaying diet-related conditions. (Dollahite et al. 2008) Positive cost-benefit analysis and willingness-to-pay ratios are important for a program with costs of \$124 - \$234 per year or \$388 per participant with a mean cost per graduate (minimum of six lessons) of \$849 (Burney & Haughton 2002, Dollahite et al. 2008). Direct outcomes of EFNEP indicate that educating decision makers could reduce food expenditures while increasing nutrition and reducing food

insecurity. EFNEP participants demonstrated the ability to reduce food expenditures, improved food planning, increased nutrition label use, increases in specific nutrients such as iron, vitamins C and B-6, and fiber, and decreases in salt use. (Burney & Haughton 2002) Research is required to analyze the effects of functional food education and its' potential health and economic impact.

### Literature Cited

- Adom, K.K.; Sorrells, M.E.; Liu, R.H. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *J. Agric. Food Chem.* **2005**. 53, 2297-2306.
- Anderson, J.W.; Hanna, T.J.; Peng X.; Kryscio R.J. Whole grain foods and heart disease risk. *J. Am. Coll. Nutr.* **2000**. 19, 291S-299S.
- Arndt, B.; Luther, M. Antioxidant Properties of Wheat Based Breakfast Foods. **2008**. In Yu, L., Wheat Antioxidants, John Wiley & Sons, Inc., Hoboken, NJ.
- Arts, I.; Hollman, P. Polyphenols and disease risk in epidemiologic studies. *J. Amer. Coll. Nutr.* **2005**. 81, 371S-325S.
- Auld, G.W.; Fulton, D. Value of theoretically based cooking classes for increasing use of commodity foods. *J. Amer. Diet. Assoc.* **1995**. 95(1), 85-86.
- Baublis, A.; Decker, E.A.; Clydesdale, F.M. Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chem.* **2000**. 68, 1-6.
- Beck, M.E. Dinner preparation in the modern United States. *British Food Journal.* **2007**. 109(7), 531-547.
- Benani, A.S.; Troy, M.C.; Carmona, X.; Fioramonti, A.; Lorsignol, C.; Leloup, L.; Casteilla, L.; Penicaud. Role for mitochondrial reactive oxygen species in brain lipid sensing. *Diabetes.* **2007**. 56, 152-160.
- Bensley, R.J.; Brusk, J.J.; Anderson, J.A.; Mercer, N.; Rivas, J.; Broadbent, L.N. wichealth.org: Impact of a stages of change-based internet nutrition education program. *J Nutr Educ Behav.* **2006**. 38, 222-229.
- Bergeson, T. National school lunch and school breakfast programs. Accessed Sept. **2008** [www.k12.wa.us/ChildNutrition/NSLSBP.aspx](http://www.k12.wa.us/ChildNutrition/NSLSBP.aspx)

- Borght, A.V.D.; Goesaert, H.; Veraverbeke, W.S.; Delcour, J.A. Fractionation of wheat and wheat flour into starch and gluten: An overview of the main processes and the factors involved. *J. Cereal Science*. **2005**. 41, 221-237.
- Burney, J.; B. Haughton. EFNEP: A nutrition education program that demonstrates cost-benefit. *J. of the Amer. Diet. Assoc.* **2002**. 102, 39-45.
- Burton-Freeman, B. Dietary fiber and energy regulation. *J Nutr.* **2000**. 130, 272S-275S.
- Cani, P.D.; Joly, E.; Horsmans, Y.; Delzenne, N.M. Oligofructose promotes satiety in healthy human: a pilot study. *European J of Clin Nutr.* **2006**. 60, 567-572.
- Carlson, A.; Mancino, L.; Lino, M. Grain consumption by Americans. Nutrition Insight 32. *USDA.CNPP*. **2005**. Accessed October 2008.  
<http://www.fns.usda.gov/cnpp/Publications/NutritionInsights/Insight32.pdf>
- Cheng, Z.; Su, L.; Moore, J.; Zhou, K.; Luther, M.; Yin, J.; Yu, L. Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *J. Agric. Food Chem.* **2006**, 54, 5623-5629.
- Cleveland, L.E.; Moshfegh, A.J.; Albertson, A.M.; Goldman, J.D. Dietary intake of whole grains. *J. Amer. Coll. Nutr.* **2000**. 19, 331S-338S.
- Coghe, S.; Benoot, K.; Delvaux, F.; Vanderhaegen, B.; Delvaux F.R. Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: Indication for feruloyl esterase activity in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2004**. 52: 602-608.
- Condrasky, M.; Graham, K.; Kamp, J. Cooking with a Chef: An innovative program to improve mealtime practices and eating behaviors of caregivers of preschool children. *J Nutr Educ Behav.* **2006**. 324-325.
- Cranfield, J.; Herath, D.; Hensen, S.; Sparling, D. An analysis of financing innovation and commercialization in Canada's functional food and Nutraceutical sector. *Agriculture and rural working paper series*. **2006**. Working paper No. 79.
- DeHaven, M.J.; Hunter, I.B.; Wilder, L.; Walton, J.W.; Berry, J. Health programs in faith-based organizations: Are they effective? *Amer. J. Public Health.* **2004**. 94(6), 1030-1036.
- Delargy, H.J.; O'Sullivan, K.R.; Fletcher, R.J.; Blundell, J.E. Effects of amount and type of dietary fibre (soluble and insoluble) on short-term control of appetite. *Int. J. Food Sci. and Nutr.* **1997**. 48, 67-77.

- Dollahite, J.; Kenkel, D.; Thompson, S.C. An economic evaluation of the expanded food and nutrition education program. *J Nutr Educ Behav.* **2008.** 40, 134-143.
- Douaud, G. Gadot prepares for US functional foods move. 2007. *Nutra-ingredients.* Accessed Nov. 21, **2007.** <http://www.nutraingredients-usa.com/news/ng.asp?id=78946-gadot-pharmline-functional-foods>.
- Dougherty, K.; Silver, C. Chef-Nutritionist team spark enjoyment and learning in cooking education series for 8- to 12-year olds. *J Nutr Educ Behav.* **2007.** 39, 237-238.
- Drewnowski, A.; Darmon, N. Food choice and diet costs: An economic analysis. *J. Nutr.* **2005.** 135, 900-904.
- Engler-Stringer, R. and Berenbaum, S. Collective kitchens in Canada: A review of the literature. *Can J Diet Prac Res.* **2005.** 65, 246-251.
- Engler-Stringer, R. and Berenbaum, S. Food and nutrition-related learning in collective kitchens in three Canadian cities. *Can J Diet Prac Res.* **2006.** 67, 178-183.
- Fang, Y.-Z.; Yang, S.; Wu, G. Free radicals, antioxidants, and nutrition. *Nutrition.* **2002.** 18, 872-879.
- FDA Whole Wheat Flour. April, **2007**, CFR 21 Section 137.200
- FDA and CFSAN. Health claim notification for whole grain foods. **1999.** 99P-2209. [www.cfsan.fda.gov/~dms/flgrains.html](http://www.cfsan.fda.gov/~dms/flgrains.html).
- Food and Nutrition Board. Proposed definition. In: *Proposed Definitions of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and Its Panel on Dietary Antioxidants and Related Compounds.* Institute of Medicine: Washington, D.C., **1998.** p 2-3.
- Frazao, E. and Allshouse J. Strategies for intervention: Commentary and debate. *Amer. Soc. Nutr. Sci.* **2003.** 133, 844S-847S.
- Frei, B. Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. *J. Nutr.* **2004.** 134, 3196S-3198S.
- Fulcher, R.G. and Rooney Duke, T.K. Whole grain structure and organization: implications for nutritionists and processors. In: "Whole Grain Foods in Health and Disease". L. Marquart, R.G. Fulcher and J.L. Slavin (Eds). American Association of Cereal Chemists, St. Paul, MN, USA **2002.** 9 – 45.

- Gallardo, G.; Jimenez, L.; Garcia-Coresa, M.T. Hydroxycinnamic acid composition and in vitro antioxidant activity of selected grain fractions. *Food Chem.* **2006**, 99, 455-463.
- Gibson, D. Food stamp program participation is positively related to obesity in low income women. *J Nutr.* **2003**, 133, 2117-2118.
- Gibson, D. Long-term food stamp program participation is positively related to simultaneous overweight in young daughters and obesity in mothers. *J Nutr.* **2006**, 136, 1081-1085.
- Grudel, S.; Garcia, A.L.; Otto, B.; Mueller, C.; Steiniger, J.; Weickert, M.O.; Speth, M.; Katz, N.; Koebnick, C. Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. *J. Nutr.* **2006**, 136, 1533-1538.
- Gruendel S.; Otto, B.; Garcia, A.L.; Wagner, K.; Mueller, C.; Weickert, M.O.; Heldwein, W.; Koednick, C. Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans. *British J Nutr.* **2007**, 98, 101-105.
- Guthrie, J.F.; Lin, B.-H.; Ploeg, M.V.; Frazao, E. Can food stamps do more to improve food choices? *Economic Information Bulletin Number 29-1*, U.S. Department of Agriculture, Economic Research Service, September **2007**, available at: <http://www.ers.usda.gov/publications/eib29/eib29-1/eib29-1.pdf>.
- Guthrie, J.F.; Stommers, E.; Voichick, J. Evaluating food stamp nutrition education: Issues and opportunities. *J Nutr Educ Behav.* **2006**, 38, 6-11.
- Gutteridge, J.M.C.; Halliwell, B. Antioxidant protection and oxygen radical signaling. In: *Reactive Oxygen Species in Biological Systems, An Interdisciplinary Approach*; Gilbert, D.L.; Colton, C.A. Kluwer Academic/Plenum Publishers: New York, **1999**, 189-212.
- Halvorsen, B.L.; Carlsen, M.H.; Phillips, K.M.; Bohn, S.K.; Holte, K.; Jacobs, D.R. Jr.; Blomhoff, R. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *Amer. J. Clin. Nutr.* **2006**, 84, 95-135.
- Harrison, G.G.; Stromer, A.; Herman D.R.; Winham. Development of a Spanish-Language version of the U.S. household food security survey module. *J. Nutr.* **2003**, 133, 1192-1197.
- Hertzler, A.A.; Bruce, F.A. Cooking, recipe use and food habits of college students and nutrition educator. *Inter. J. Consumer Studies.* **2002**, 26(4), 340-345.



- Hoad, C.L.; Rayment, P.; Spiller, R.C.; Marciani, L.; Alonso, B.C.; Traynor, C.; Mela, D.J.; Peters, H.P.F; Gowland, P.A. In vivo imaging of intragastric gelation and its effect on satiety in humans. *J. Nutr.* **2004**. 134, 2293-2300.
- Holt, S.H.A.; Brand-Miller, J.C.; Stitt, P.A. The effects of equal-energy portions of different breads on blood glucose levels, feelings of fullness and subsequent food intake. *J Am Diet Assoc.* **2001**. 101, 767-773.
- House Committee on Agriculture. 2008 Farm Bill Nutrition Title: Promoting Health and Fighting Hunger in the United States. Accessed September 2008.  
[http://agriculture.house.gov/inside/Legislation/110/FB/Conf/Title\\_IV\\_fs.pdf](http://agriculture.house.gov/inside/Legislation/110/FB/Conf/Title_IV_fs.pdf)
- Howarth, N.C.; Saltzman, E.; McCrory, M.A.; Greenberg, A.S.; Dwyer, J.; Ausman, L.; Kramer, D.G.; Roberts, S.B. Fermentable and nonfermentable fiber supplements did not alter hunger, satiety or body weight in a pilot study of men and women consuming self-selected diets. *J. Nutr.* **2003**. 133, 3141-3144.
- Howarth, N.C.; Huang, T. T.-K.; Roberts, S.B.; McCrory, M.A. Dietary fiber and fat are associated with excess weight in young and middle-age US adults. *J Am Diet Assoc.* **2005**. 105, 1365-1372.
- Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**. 50, 1815-1821.
- International Food Information Council. Functional Foods. Accessed November 2007.  
<http://www.ific.org/nutrition/functional/upload/functionalfoodsbackgrounder.pdf>
- Jenkins, D.-J.A.; Kendall, C.-W.C. Plant sterols, health claims, and strategies to reduce cardiovascular disease risk. *J. of the Amer. Coll. Nutr.* **1999**. 18(6), 559-562.
- Jones, J.M.; Reicks, M.; Adams, J.; Fulcher, G.; Weaver, G.; Kanter, M.; Marquart, L. The importance of promoting a whole grain foods message. *J Amer Coll Nutr.* **2002**. 21(4), 293-297.
- Johnston, K.P.; Sharp, M.; Clifford; Morgan, L. Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. *FEBS Letters.* **2005**. 579, 1653-1657.

- Johnson-Taylor, W.L.; Yaroch, A.L.; Krebs-Smith, S.M.; Rodgers, A.B. What can communication science tell us about promoting optimal dietary behavior? *J Nutr Educ Behav.* **2007**. 39, S1-S4.
- Katina, K.; Arendt, E.; Liukkonen, K.-H.; Autio, K.; Flander, L.; Poutanen, K. Potential of sourdough for healthier cereal products. *Trends in Food Science & Technology*, **2005**, 16, 104-112.
- Kempson, K.; Keenan, D.P.; Sadani, P.S.; Adler A. Maintaining food sufficiency: Coping strategies identified by limited-resource individuals versus nutrition educators. *J. Nutr. Educ. Behavior.* **2003**. 35, 179-188.
- Kimm, S.Y.S. The role of dietary fiber in the development and treatment of childhood obesity. *Pediatrics.* **1995**. 1010-1014.
- Klaus, S.; Pultz, S.; Thone-Reineke, C.T.; Wolfram, S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Inter. J. Nutr.* **2005**. 29, 615-623.
- Klohe-Lehman, D.; Freelang-Groves, J.; Anderson, E.R.; McDowell, T.; Clarke, K.K.; Hanns-Nuss, H.; Cai, G.; Puri, D.; Milani, T.J. Nutrition knowledge is associated with greater weight loss in obese and overweight low-income mothers. *J. Amer. Diet. Assoc.* **2006**. 106, 65-75.
- Koh-Banerjee, P. and Rimm, E.B.. Whole grain consumption and weight gain: a review of the epidemiological evidence, potential mechanisms and opportunities for future research. *Proceeding of the Nutrition Soc.* **2003**. 62, 25-29.
- Kobayashi, Y.; Suzuki, M.; Satsu, H.; Arai, S.; Hara, Y.; Suzuki, K.; Miyamoto, Y.; Shimizu, M. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J. Agric. Food Chem.* **2000**. 48: 5618-5623.
- Koebnick, C.; Gruendal, S.; Garcia, A.L.; Otto, B.; Weickert, M.O. Carob pulp preparation rich in insoluble fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin. *FASEB.* **2006**. 20(4), A599.
- Kris-Etherton, P.M.; Hecker, K.D.; Bonanome, A.; Coval, S.M.; Binoski, A.E.; Hilpert, K.F.; Griel, A.E.; Etherton, T.D. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med.* **2002**. 113(9B), 71S-88S.
- Landers, P.S. The Food Stamp Program: History, nutrition education, and impact. *J. Am Diet Assoc.* **2007**. 107, 1945-1951.

- Lang, R.; Thane, C.W.; Bolton-Smith, C.; Jebb, S.A. Consumption of whole-grain foods by British adults: findings from further analysis of two national dietary surveys. *Public Health Nutr.* **2002**. 5, 479-484.
- Larson, N.I.; Perry, C.L.; Story, M.; Neumark-Sztainer D. Food preparation by young adults is associated with better diet quality. *J. Amer. Diet. Assoc.* **2006a**. 106, 2001-2007.
- Larson, N.I.; Story, M.; Eisenberg, M.E.; Neumark-Sztainer D. Food preparation and purchasing roles among adolescents: Associations with sociodemographic characteristics and diet quality. *J. Amer. Diet. Assoc.* **2006b**. 106, 211-216.
- Leenhardt, F.; Lyan, B.; Rock, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesy, C. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *J. Agric. Food Chem.* **2006**. 54, 1710-1715.
- Levy, J.; Auld, G. Cooking classes outperform cooking demonstrations for college sophomores. *J Nutr Educ Behav.* **2004**. 36, 197-203.
- Li, W.; Pickard, M.D.; Beta, T Effect of thermal processing on antioxidant properties of purple wheat bran. *Food Chem.* **2007**. 104, 1080-1086.
- Little, D.M.; Newman, M.E. **2002**. Cooperative Extension/Land-Grant University System Food Stamp Nutrition Education National Report. USDA/CREES. Accessed Oct. 12, 2008.  
[http://www.csrees.usda.gov/nea/food/fsne/pdfs/fsne\\_national\\_report\\_2002.pdf](http://www.csrees.usda.gov/nea/food/fsne/pdfs/fsne_national_report_2002.pdf)
- Liyana-Pathirana, C.M.; Shahidi, F. Antioxidant properties of commercial soft and hard winter wheat (*Triticum aestivum* L.) and their milling fractions. *J. Sci. Food & Ag.* **2006**. 86(3), 477-485.
- Liyana-Pathirana, C.M.; Shahidi, F. Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chem.* **2007**. 101, 1151-1157.
- Lindenmeier, M.; Hofmann, T. Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *J. Agric. Food Chem.* **2004**, 52, 350-354.
- Lopez, H.W.; Krespine, V.; Guy, C.; Messenger, A.; Demigne, C.; Remesy, C. Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. *J. Agric. Food Chem.* **2001**, 49, 2657-2662.
- Marquart, L.; Wiemer, K.L.; Jones, J.M.; Jacob, B. Whole grain health claims in the USA and other efforts to increase whole-grain consumption. *Proceedings of the Nutrition Society.* **2003**. 63: 151-160.

- Marquis, S.; Thomson, C.; Murray, A. Assisting people with a low income to start and maintain their own community kitchens. *Can J Diet Prac Res.* **2001.** 62, 130-132.
- McCabe, B.J.; Copeland, N.P.; Shull, C.A.; Khandra, D.D. Telehealth and Telenutrition: The use of interactive compressed video for nutrition counseling of rural elderly. *J. Nutr. for the Elderly* **2001.** 21(2), 73-83.
- McDougall, G.J.; Stewart, D. The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* **2005.** 2, 189-195.
- McLaughlin, C.; Tarasuk, V.; Kreiger, N. An examination of at-home food preparation activity among low-income, food-insecure women. *J. Amer. Diet. Assoc.* **2003.** 103(11), 1506-1512.
- Melanson, K.J.; Angelopoulos, T.J.; Nguyen, V.T.; Martini, M.; Zukley, L.; Lowndes, J.; Dube, T.J.; Fiutem, J.J.; Yount, B.W.; Rippe, J.M. Consumption of whole-grain cereals during weight loss: Effects on dietary quality, dietary fiber, magnesium, vitamin B-6, and obesity. *J Am Diet Assoc.* **2006.** 106, 1380-1388.
- Mennella, J.A.; Ziegler, P.; Briefel, R.; Novak, T. Feeding infants and toddlers study: The types of foods fed to Hispanic infants and toddlers. *Amer. Diet. Assoc.* **2006.** 106, S96-S105.
- Michaud, P.; Condrasky, M.; Griffin, S.F. Review and application of current literature related to culinary programs for nutrition educators. *Top Clin Nutr.* **2007.** 22, 336-348.
- Miller, H.E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *J of the Amer. Coll. Nutr.* **2000.** 19(3), 312S-319S.
- Mintel Global New Products Database, "Global New Product Database, Monitoring New Trends and Innovation. <http://www.gnpd.com/sinatra/gnpd/frontpage/>. **2007.**
- Moore, J.; Hao, J.; Zhou, K.; Luther, M.; Costa, J.; Yu L. Carotenoid, tocopherols, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005.** 53(17), 6649-6657.
- Moore, J.; Cheng, Z.; Hao, J.; Guo, G.; Liu, J.-G.; Lin, C.; Yu, L. Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *J. Agric. Food Chem.* **2007.** 55, 10173-10182.

- Moore, J.; Cheng, Z.; Su, L.; Yu, L. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *J. Agric. Food Chem.* **2006**, 54, 9032-9045.
- Moore, J.; Yu, L. Methods for antioxidant capacity estimation of wheat and wheat-based food products. In: *Wheat Antioxidants*. Yu, L. John Wiley & Sons, Inc. Hoboken, New Jersey, **2008**, pp 118-173.
- Nandakumar, V.; Singh, T.; Katiyar, S.K. Multi-targeted prevention and therapy of cancer by proanthocyanidins. *Cancer Letters*. **2008**, 269, 378-387.
- Ou, S-Y, GM Jackson, X Jiao, J Chen, J-Z Wu, and X-S Huang. Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. *J. Agric. Food Chem.* **2007**, 55: 3191-3195.
- Padayatty, S.J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.-H.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S.K.; Levine, M. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J Amer Coll Nutr.* **2003**, 22(1), 18-35.
- Pai, S.; Ghugre, P.S.; Udipi, S.A. Satiety from rice based, wheat based, and rice-pulse combination preparations. *Appetite*. **2005**, 44, 263-271.
- Park, H.; Seib, P.A.; Chung, O.K.; Seitz, L.M. Fortifying bread with each of three antioxidants. *Cereal Chem.* **1997**, 74, 202-206.
- Pasman, W.J.; Saris, W.H.M; Wauters, M.A.J.; Westerterp-Plantenga, M.S. Effect of one week of fibre supplementation on hunger and satiety ratings and energy intake. *Appetite*. **1997**, 29, 77-87.
- Pereira, MA, and S Liu. Types of carbohydrates and risk of cardiovascular disease. *J Women's Health*. **2003**, 12(2), 115-122.
- Polidori, M.C. Antioxidants micronutrients in the prevention of age-related diseases. *J Postgrad Med.* **2003**, 49, 229-235.
- Ranhotra, G.S.; Gelroth, J.A.; Langemeier, J.; Rogers, D.E. Stability and contribution of beta carotene added to whole wheat bread and crackers. *Cereal Chem.* **1995**, 72, 139-141.
- Rimm, E.B.; Katan, M.B.; Ascherio, A.; Stampfer, M.J.; Willett, W.C. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med.* **1996**, 125, 384-389.

- Rose, D. Food stamps, the thrifty food plan, and meal preparation: The importance of the time dimension for US nutrition policy. *J Nutr Educ Behav.* **2007.** 39, 226-232.
- Rosenbaum, D. Farm bill contains significant domestic nutrition improvements. <http://www.cbpp.org/5-8-08fa.htm>. Sept. **2008** Center on Budget and Policy Priorities.
- Ross, A.B.; Kamal-Eldin, A.; Aman, P. Dietary Alkylresorcinols: Absorption, bioactives, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr. Rev.* **2004,** 62, 81-95.
- Samra, R.A.; Anderon, G.H. Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men. *Am J Clin Nutr.* **2007.** 86, 972-929.
- Salmenkallio-Marttila M, Katina K, Autio K. Effects of bran fermentation on quality and microstructure of high-fiber wheat bread. *Cereal Chem.* **2001.** 78(4), 429-435.
- Schneeman, B.O. Fiber, inulin, and oligofructose: similarities and differences. *J. Nutr.* **1999.** 129, 1424S-1427S.
- Seidell, J.C. Epidemiology and health economics of obesity. *Medicine.* **2003.** 5-7.
- Shahidi, F. Functional foods: Their role in health promotion and disease prevention. *J. Food Sci.* **2004.** 69, 146-149.
- Shahidi, F. & Ho, C.T. *Antioxidant Measurement and Applications.* ACS Symposium Series 956. Oxford University Press: Washington, D.C. **2005.**
- Slavin, J.L. Mechanisms for the impact of whole grain foods and cancer risk. *J. Amer.Coll. Nutr.* **2000.**19(3), 300S-307S.
- Slavin, J.L.; Jacobs, D.; Marquart, L. Grain processing and nutrition. *Crit. Rev. in Biotech.* **2001.** 21, 49-66.
- Slavin, J. Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society.* **2003,** 62, 129-134.
- Stanner, S.A.; Hughers, J.; Kelly, C.N.M.; Buttriss, J. A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutr.* **2003.** 7(3), 407-422.

- Stead, M.; Caraher, M.; Wreiden, W.; Longbottom, P.; Valentine, K.; Anderson, A. Confident, fearful and hopeless cooks: Findings from the development of a food-skills initiative. *British Food Journal*. **2004**. 106(4), 274-287.
- Steinhubl, S.R. Why have antioxidants failed in clinical trials? *Am J Cardiol*. **2008**. 101, 14D-19D.
- Strobel, P.; Allard, C.; Perez-Acle, T.; Calderon, R.; Aldunate, R.; Leighton, F. Myricetin, quercetin, and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. *Biochem. J*. **2005**. 386, 471-478.
- Swinburn, B.; Caterson, I.; Seidell, J.C.; James, W.T.P. Diet, nutrition and the prevention of excess weight gain and obesity. *Public Health Nutrition*. **2004**. 7(1A), 123-146.
- Taylor, M.R.; Brester, G.W.; Boland, M.A. Hard white wheat and gold medal flour: General Mills' contracting program. *Review of Ag. Economics*. **2005**. 27(1), 117-129.
- Taylor-Powell, E. Evaluating food stamp nutrition education: A view from the field of program evaluation. *J Nutr Educ Behav*. **2006**. 12-17.
- The Whole Grains Council, "A Consumer Definition of Whole Grains." <http://www.wholegrainscouncil.org/>. **2006**.
- The Whole Grain Council. 2007. Whole Grain Facts and Figures. *The Whole Grains Council*. Accessed Nov.20, 2007. <http://www.wholegrainscouncil.org/WGFacts+Figures.htm>.
- Thompson, W.G.; Holdman, N.R.; Janzow, D.J.; Slezak, J.M.; Morris, K.L.; Zemel, M.B. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. *Obesity Research*. **2005**. 13(8), 1344-1352.
- Title 21 Code of Federal Regulation. (21 CFR) Flour 137.109.
- Title 21 Code of Federal Regulation. (21 CFR) Whole Wheat Flour 137.200.
- Title 21 Code of Federal Regulation. (21 CFR) Whole Durum Flour 137.225.
- Townsend, M.S. Evaluating food stamp nutrition education: Process for development and validation of evaluation measures. *J Nutr Educ Behav*. **2006**. 38, 18-24.
- Tsuneki, H.; Ishizuka, M.; Terasawa, M.; Wu, J.-B.; Sasaoka, T.; Kimura, I. Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. *BMC Pharmacology*. **2004**. 4:18.

- US Food and Drug Administration. (2006). Guidance for Industry and FDA Staff: Whole Grains Label Statements. C15 AACC International Task Force. Retrieved November 9, 2008 from USDA at <http://www.cfsan.fda.gov/~dms/flgragui.html>
- USDA. Cooperative State Research, Education, and Extension Service. (2008). Expanded Food, Nutrition and Education Program. Accessed October 15, 2008. <http://www.csrees.usda.gov/nea/food/efnep/efnep.html>
- USDA. Economic Research Services. (2007). Food Availability (Per Capita) Data System: Food Guide Pyramid. Accessed November 21, 2007. <http://www.ers.usda.gov/Data/FoodConsumption/FoodGuideIndex.htm#grain>.
- USDA. Economic Research Services. (2008). Wheat: Planted acreage, harvested acreage, production, yield, and farm price. Accessed November 21, 2007. <http://www.ers.usda.gov/Data/Wheat/YBtable01.asp>
- U.S. Departments of Health and Human Services and U.S. Department of Agriculture.(2005). Dietary Guidelines for Americans, 2005. 6<sup>th</sup>. Edition, Washington, DC: U.S. Government Printing Office.
- Vivekananthan, D.P.; Penn, M.S.; Sapp, S.K.; Hsu, A.; Topol, E.J. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. *Lancet*. **2003**. 361, 2017-2023.
- Warfel, K.; Aso, Y.; Gee, D.L. Regulation of functional foods in Japan: Foods for Specialized Health Uses (FOSHU). *J. of the Amer. Diet. Assoc.* **2007**. 107(8): A34.
- Wattanapitayakul, S.K.; Bauer, J.A. Oxidative pathways in cardiovascular disease roles, mechanisms, and therapeutic implications. *Pharmacology & Therapeutics*. **2001**. 89, 187-206.
- Webb, A.L.; Schiff, A.; Currivan, D.; Villamor, E. Food stamp program participation but not food insecurity is associated with higher adult BMI in Massachusetts residents living in low-income neighborhoods. *Public Hlth Nutr.* Published online by Cambridge University Press May **2008**. doi:10.1017/S1368980008002309.
- Weickert, M.O.; Spranger, J.; Holst, J.J.; Otto, B.; Koebnick, C.; Mohlig, M.; Pfeiffer, A.F.H. Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety. *B J Nutr.* **2006**. 96, 795-798.



- Wennermark, B.; Jagerstad, M. Breadmaking and storage of various wheat fractions affect vitamin E. *J. Food Sci.* **1992**, *57*, 1205-1209.
- Wilcox, B.J.; Curb, J.D.; Rodriguez, B.L.; Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies. *Am J Cardiol.* **2008**. 101, 75D-86D.
- Wilson, B.J. Designing media messages about health and nutrition: What strategies are most effective? *J Nutr Educ Behav.* **2007**. 39. S13-S19.
- Wreiden, W.L.; Anderson, A.S.; Longbottom, P.J.; Valentine, K.; Stead, M.; Caraher, M.; Lang, T.; Gray, B.; Dowler, E. The impact of a community-based food skills intervention on cooking confidence, food preparation methods and dietary choices – an exploratory trial. *Public Health Nutrition*, **2007**. 10(2), 203-211.
- Yu, L.; Haley, S.; Perret, J.; Harris, M. Comparison of wheat flours grown at different locations for their antioxidant properties. *Food Chem.* **2004a**, *86*, 11-16.
- Yu, L.; Perret, J.; Harris, M.; Wilson, J.; Haley, S. Antioxidant properties of bran extracts from “Akron” wheat grown in different locations. *J Agric Food Chem.* **2003**. 56(24), 1566-1570.
- Yu, L.; Zhou, K. Antioxidant properties of bran extracts from 'Platte' wheat grown at different locations. *Food Chem.* **2004b**, *90*, 311-16.
- Zhou, K.; Su, L.; Yu, L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004a**, *52*, 6108-6114.
- Zhou, K.; Laux, J. J.; Yu, L. Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. *J. Agric. Food Chem.* **2004b**, *52*, 1118-1123.
- Zhou, K.; Yu, L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem.* **2004c**, *52*, 1112-1117.

## Chapter 2: Effect of Yeast Strain on the Antioxidant Properties of Wheat Bran Following Solid-State Treatments

### Abstract

Wheat bran contains a significant portion of the bioactive components in wheat. This study assessed the ability of 10 food grade yeasts to increase the extractable antioxidant activity of wheat bran in order to increase its health beneficial properties. Bran samples were treated with yeasts at two doses,  $5.6 \times 10^4$  and  $1.8 \times 10^5$  yeast cells per g wheat bran, for low and high doses, respectively. Treated bran samples were then analyzed for their ability to increase antioxidant capacity when compared with an inactivated control of yeast and a blank of sterile water over 72 h at ambient temperature. Under the specified conditions yeast strain LBE.11 showed the greatest potential to increase antioxidant capacity, increasing ORAC and HOSC radical scavenging by 50% and 67%, respectively. Results suggest the possibility to develop viable postharvest methods for enhancement of wheat bran health properties.

### Introduction

Epidemiological studies show that a diet rich in foods containing antioxidants such as fruits, vegetables, and other plant derived foods decreases the risk of certain chronic diseases (Wattanapitayakul & Bauer 2001, Stanner et al. 2003, Wilcox et al. 2008). Antioxidants broadly include “any substance that when presented at low concentrations, compared to those of an oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate” (Gutteridge & Halliwell 1999). The

physiological relevance of antioxidants is their protection from free radicals, molecules with an unpaired electron in the outer orbital shell, termed the oxidative defense mechanism which prevents damage to biomolecules (Wilcox et al. 2004, Fang et al. 2002, Wilcox et al. 2008).

Wheat comprises one-third of the total grain crop world wide (Slavin 2001). At 117.5 grams per day, wheat based products account for over 70% of the total U.S. grain intake (USDA 2007). A multitude of antioxidant compounds have been found in wheat including phenolic acids, flavonoids, coumarins, polyphenols, phytates, terpenes, carotenoids, tocopherols, and tocotrienols (Shahidi 2004). When tested against free radical systems, wheat and wheat bran extracts have demonstrated scavenging of the DPPH, ABTS cation, peroxy, superoxide anion, and hydroxyl radicals, inhibition of oxidation of fish and corn oil, human low-density lipoprotein cholesterol and DNA, and chelation of transition metal  $\text{Fe}^{2+}$  (Yu et al. 2002a, Yu et al. 2002b, Yu et al. 2004, Zhou & Yu 2004, Zhou et al. 2004b, Moore et al. 2005, Yu et al. 2005, Liyana-Pathirana & Shahidi 2006, Moore et al. 2006a, Anson et al. 2008). The majority of these compounds and radical scavenging capacity is located in the bran fraction (Zhou et al. 2004b, Liyana-Pathirana & Shahidi 2006). Should whole grain consumption continue to increase, enhancing the bioavailability of these antioxidants through post harvest treatment may increase the overall healthfulness of whole grain foods.

Fermentation of agricultural commodities with bacteria, mold, and yeasts has been investigated to develop functional ingredients by increasing bioavailability of pre-existing bioactive compounds or enrichment through metabolism products

(Bartolome et al. 2003, Fernandez-Orozco et al. 2007, Moore et al. 2007, Lee et al. 2008). Increased bioavailability is possible through the use of enzymes, including those produced by microorganisms, such as xylanases, arabinase,  $\alpha$ -L-arabinosidase,  $\beta$ -glucuronases, and cellulases that are predicted to break the ester link between phenolic acids and cell wall materials (Bartolome et al. 2003, Moore et al. 2006a). Solid state fermentation of cassava peels and wheat bran demonstrated the potential for protein enriched food ingredients (Oboh 2006, Moore et al. 2007).

The capacity of a select strain to enhance the nutritive or mechanical properties of raw commodities is well known (Wang and Fields, 1978; Ejiofor et al 1996, Mathew and Abraham 2005, Plessas et al 2007). To date, no known study has been conducted to analyze the effect of multiple commercially available food grade strains of yeast under industry relevant solid state conditions. Yeasts naturally contain cellular antioxidant defenses under stressful conditions. Antioxidant activities and activities of catalase and superoxide dismutase (SOD), the associated enzymes, were found to be yeast strain specific, either increasing or decreasing, when adapting to hydrogen peroxide exposure (Bayliak et al. 2006). The aim of the present work was to study the effect yeast strain and dose on the extractable antioxidant activities of soft red wheat bran.

## Materials and Methods

### **Chemicals & Reagents**

Wheat bran was generously donated by The Mennel Milling Company (Fostoria, OH). 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), disodium ethylenediaminetetracetate, and 2,2'-bipyridyl gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals, USA, Inc. (Richmond, VA). Petrifilm Yeast and Mold plates were purchased from 3M (St. Paul, MN). YPD broth and agar were purchased from Fisher Scientific and prepared as directed (Pittsburg, PA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

### **Yeast & Wheat Bran Samples**

Our laboratory has recently established a library of the commercially available yeasts, including those traditionally used for baking, beer, and wine production. Yeasts were streaked onto YPD plates to remove additives and ensure single strain selection. Log growth studies were conducted for 80 strains of yeast to determine the time frame that the yeast strains are actively growing under selected conditions (See Appendix a). These log growth cycle tests demonstrated a consistent window of time in which yeast strains were in logarithmic growth. Multiple strains were then combined and run over duplicate dates to ensure the period of exponential growth was not altered significantly by different ages of yeasts. Ten yeasts samples were

selected for solid state treatment. These included: Hodgson Mill All Natural Active Dry Yeast – Whole Grain, Saf-instant Instant Yeast, Sourdoughs International Italian (Camaldoli) Sourdough Starter, Wyeast Bavarian Wheat Blend, Brewferm Blanche, Lallemand Diamond Lager Yeast, Saflager S-04, BM DYW74, Lalvin D 47, and Red Star Cote des Blancs labeled as LBR.04, LBR.08, LBR.21, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17, respectively. Bran was ground to a 40 mesh size using a 3600 Laboratory Mill from Perten Instruments (Huddinge, Sweden).

### **Yeast Preparation**

Individual yeast strains were used to inoculate 10 mL YPD broth and grown at 30 °C for 48 hrs. One loop of yeast cells was transferred to 10 mL fresh YPD broth and incubated for a second 48 hrs. at 30 °C. 200 µL were transferred to 50 mL of YPD broth and grown to the pre-determined log phase. Yeasts were centrifuged 2 minutes at 2000 rpm at 30 °C, the supernatant decanted and yeast cells washed with 10 mL sterile water at 30 °C. The optical density was then adjusted with sterile water to an absorbance of 0.5 at 600 nm. Yeast dosages will be prepared with the high dose being 2 mL of 0.5 O.D. yeast preparation, a low dose of 1 mL 0.5 O.D. yeast preparation and 1 mL sterile water, and a control of 2 mL of the high dose inactivated by microwave.

### **Yeast Counts**

Yeast counts were conducted on the yeast treatments prior to the solid state treatment and on the treated wheat bran following the solid state treatment. For the

yeast treatments, serial dilutions of the high, low, and control were made using sterile water. For post-treatment counts, 0.01 g of solid state yeast treated bran were weighed out and used for a serial dilution series. One mL of each serial dilution was plated according to manufacturers' directions on 3M yeast and mold count petrifilm. Yeast counts were read after 5 days of incubation at 20 °C.

### **Solid State Yeast Treatment**

Soft red winter wheat bran was ground to pass through a #40 mesh and 5 g measured into sterile 50 mL beakers. A total volume of 2 mL yeast treatment was added to the bran, while simultaneously being stirred gently for 15 seconds. Yeast treatments were conducted in triplicate in addition to a negative control containing 5 g bran and 2 mL of sterile water. Beakers were covered with parafilm and incubated 72 hours at ambient temperature. Following incubation, 0.01 g treated wheat bran was removed for post-reaction yeast count. The remaining solid state treated bran thermally inactivated by microwave, ground to pass through a #40 mesh and stored at -80 °C until further analysis.

### **Sample Extraction Procedures**

Samples re-ground following treatment to a uniform #40 mesh size were extracted using 50 % acetone at a ratio of 1 gram material (dry weight basis) per 10 mL solvent, overnight. Samples were centrifuged and the supernatants collected. Samples were stored under nitrogen in the dark at 20 °C until further analysis.

### **Total Phenolic Content**

Total phenolic content was measured using the Folin-Ciocalteu reagent according to a laboratory procedure (Yu et al 2002a). The final reaction mixture contained 100  $\mu$ L of sample extract, 500  $\mu$ L of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate and 1.5 mL pure water. Absorbance at 765 nm was recorded after two hours incubation at ambient temperature. Gallic acid equivalence was calculated using a gallic acid standard curve. TPC was determined in duplicate for each sample and then samples of the same yeast and treatment combined for analysis (n = 6).

### **Radical Cation ABTS $\bullet^+$ Scavenging Capacity**

Sample extracts were tested against the ABTS $\bullet^+$  cation radical generated according to a laboratory protocol (Miller, Rice-Evans 1996, Zhou and Yu 2004). Briefly, an ABTS $\bullet^+$  solution was prepared by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide for 30 minutes at ambient temperature. The solution was then filtered and adjusted with phosphate buffer to an absorbance of 0.7 at a wavelength of 734 nm. A trolox standard is prepared with the same solvent with the blank consisting of 1 mL phosphate buffer and 80  $\mu$ L water blank. Likewise the final reaction mixture contained 1 mL of the ABTS $\bullet^+$  solution, and 80  $\mu$ L of the sample of trolox standard. Reaction mixtures were vortexed 30 seconds and a reading recorded at 1.5 minute. Data is reported as  $\mu$ moles of trolox equivalents per gram solid state yeast treated bran calculated using the trolox standard curve.



### **Oxygen Radical Absorbance Capacity (ORAC) Assay**

ORAC was conducted on sample extracts using the fluorescent probe fluorescein (FL) according to a laboratory protocol (Huang et al 2002, Zhou and Yu 2004). Trolox standards were prepared in the same solvent as the samples and AAPH and FL solutions were prepared with 75 mM phosphate buffer (pH 7.4). The final reaction mixture per well contained 225  $\mu$ L of 81.63 nM FL solution, 30  $\mu$ L blank (50% acetone), standard, or sample solution, and 25  $\mu$ L 0.36 M AAPH solution. Therefore, each well contained a constant total volume of 280  $\mu$ L. Standards and samples were run in duplicate simultaneously utilizing a Victor<sup>2</sup> multilabel counter (PerkinElmer, Turku, Finland). The reaction mixture was held at 37 °C and fluorescence measurements taken every minute at excitation and emission wavelengths of 485 nm and 530 nm respectively for 1.5 hours. Results are expressed as trolox equivalents (TE) calculated using the relative area under the curve for the samples as compared to the trolox standard curve run simultaneously. Samples of the same yeast and dose treatment were combined and reported as the mean (n = 6) and standard deviation.

### **Hydroxyl Radical Scavenging Capacity (HOSC) Assay.**

Hydroxyl radical scavenging capacity is measured using by the hydroxyl radical generated by ferric iron ( $\text{Fe}^{3+}$ ) and hydrogen peroxide as described previously (Moore et al. 2006b). Briefly a 92.8 nM solution of fluorescein (FL) was prepared

using phosphate buffer. The final well concentration was 300  $\mu\text{L}$  per well consisting of 170  $\mu\text{L}$  FL solution, 30  $\mu\text{L}$  blank, standard, or sample, 40  $\mu\text{L}$   $\text{H}_2\text{O}_2$  and 60  $\mu\text{L}$   $\text{FeCl}_3$ . Degradation of the FL solution is measured every minute using a Victor<sup>2</sup> multilabel counter (PerkinElmer, Turku, Finland). Trolox equivalents are calculated using the area under the curve as compared with the trolox standard run simultaneously with the samples. Data is reported as the mean ( $n = 6$ ) of each treatment and standard deviations.

### **Radical DPPH Scavenging Capacity.**

Free radical scavenging capacity of each sample was estimated following a previously reported procedure utilizing the stable 2,2-diphenyl-1-picrylhydrazyl radical ( $\text{DPPH}^\bullet$ ) (Yu et al. 2002b), by the high-throughput radical scavenging capacity assay (RDSC) (Moore et al. 2006a). The 200  $\mu\text{M}$   $\text{DPPH}^\bullet$  working solution was freshly prepared from a 1 mM  $\text{DPPH}^\bullet$  stock solution in sample solvent. Briefly, 100  $\mu\text{L}$  of freshly made 200  $\mu\text{M}$   $\text{DPPH}^\bullet$  working solution was combined with 100  $\mu\text{L}$  of solid state yeast treatment extract to start the radical-antioxidant reaction. On the same plate was also a blank of pure sample solvent, and control of solvent and 200  $\mu\text{M}$   $\text{DPPH}^\bullet$  working solution, and standards ranging from 7.8 to 250 mM Trolox. The absorbance at 517 nm was determined by Victor<sup>3</sup> multilabel plate reader over 40 minutes (Perkin-Elmer, Turku, Finland). The total area under the curve was calculated and used to determine the % DPPH radical quenched and RDSC value (TE mmol/g). Samples were run in duplicate and treatments of the same yeast and dose combined for reporting the mean ( $n = 6$ ) and standard deviation.

## Statistical Analysis

Data are reported as mean  $\pm$  SD of the combined treatments with individual extracts run in duplicate. Thus, data for the solid state treatment, pizza crust, and in-vitro digestion model will be the mean  $\pm$  SD for six values. One-way Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) tests were performed (Statistical Package for the Social Sciences (SPSS) for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to identify differences among means, with statistical significance declared at  $P \leq 0.05$ .

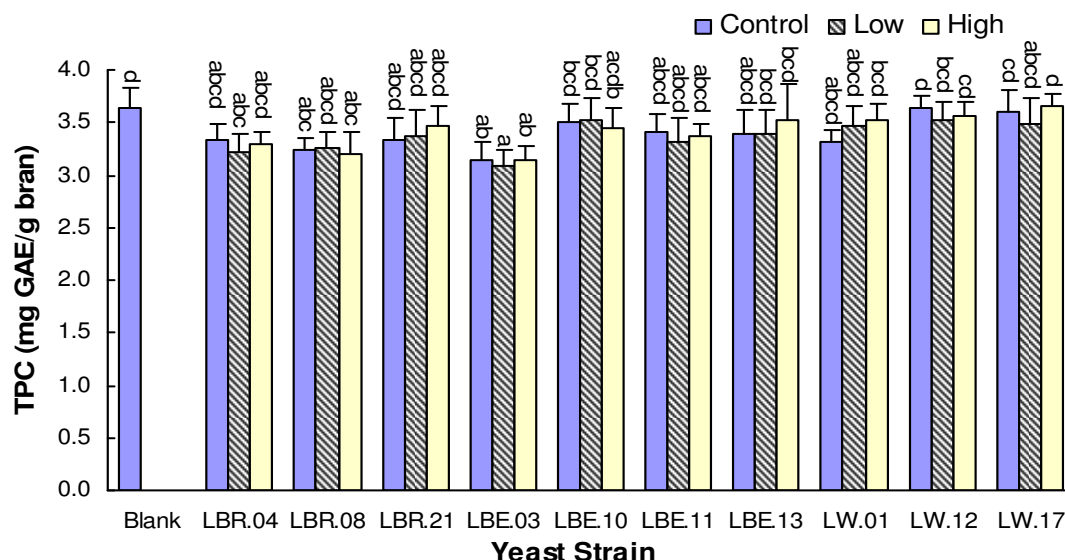
## Results and Discussion

*Effects of Different Yeast Strains on the Antioxidant Properties of Soft Red Wheat Bran Under Solid State Treatment Conditions.* Solid state yeast treatments were conducted with 10 food grade yeasts (3 traditionally used for bread, 4 for beer, and 3 for wine) at two dose levels and compared with their controls, thermally inactivated high doses of the same yeast, and a negative control of sterile water which were all run under the same conditions. The ten yeasts used are represented as LBR.04, LBR.08, LBR.21, LBE.03 LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 in correspondence with their library identification. Strains beginning with LBR, LBE, and LW are traditionally used in bread production, beer, and wine, respectively. Solid-state treatment conditions were selected to test the selected strains under industry possible conditions. Under these conditions, there were few

differences between yeast strains and their abilities increase the extractable antioxidant components of soft red winter wheat bran.

*Yeast Counts* – All controls were negative for yeast and mold. Low doses of yeast contained approximately  $2.8 \times 10^5$  cells per mL or  $5.6 \times 10^4$  cells per g. High doses of yeast contained approximately  $9.0 \times 10^5$  cells per mL or  $1.8 \times 10^5$  cells per g. Following solid state treatment there was no significant difference in yeast count between yeast strain for low and high doses. Low and high solid state yeast treatment wheat bran samples contained approximately  $4.5 \times 10^4$ , and  $1.0 \times 10^5$  cells per g treated wheat bran, respectively.

*Total Phenolic Content (TPC)* – **Figure 2.1** shows the total phenolic content of the controls and their corresponding yeast treatments. Low and high dose yeast treatments under solid state conditions were not significantly different from their corresponding control. It was interesting to note that the controls when analyzed independently differed significantly indicating that yeast themselves may differ in their extractable TPC. The same observation was made previously in inactivated yeast controls in Lakin wheat bran (Moore et al. 2007).

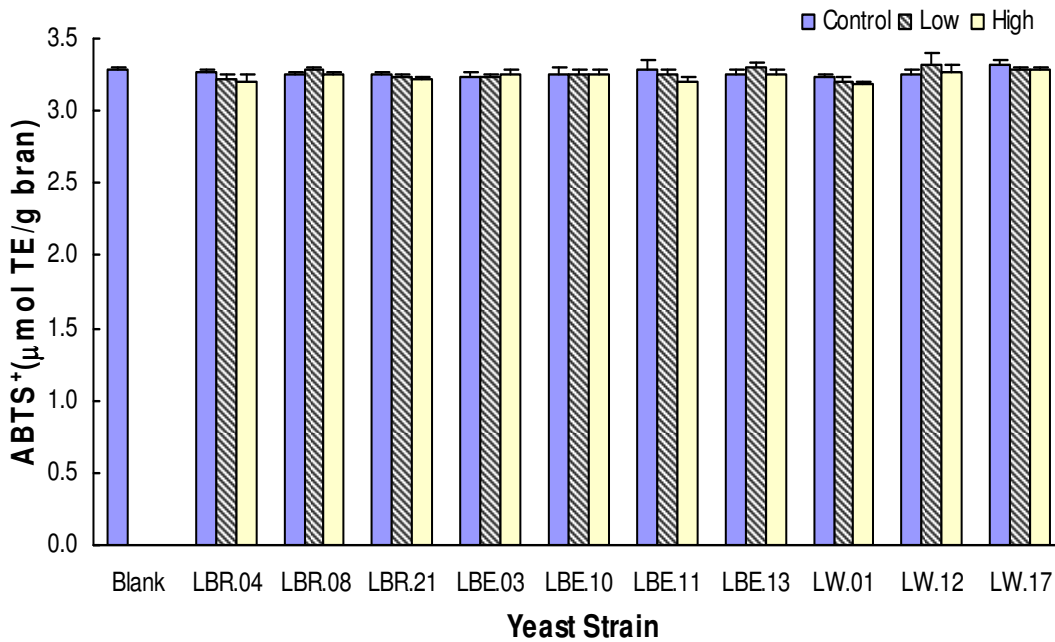


**Figure 2.1 Effect of solid state yeast treatment on the total phenolic content (TPC) of soft red wheat bran.** Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of yeast treated wheat bran on a dry weight basis. Blank represents wheat bran run concurrently with sterile water. LBR.04, LBR.08, LBR.21, LBE.03, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 represent food grade yeasts from our laboratory yeast library. Control is inactivated yeasts; low and high were treated with  $5.6 \times 10^4$  and  $1.8 \times 10^5$  cells per g wheat bran, respectively at ambient temperature for 72 h. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported (n = 6). Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

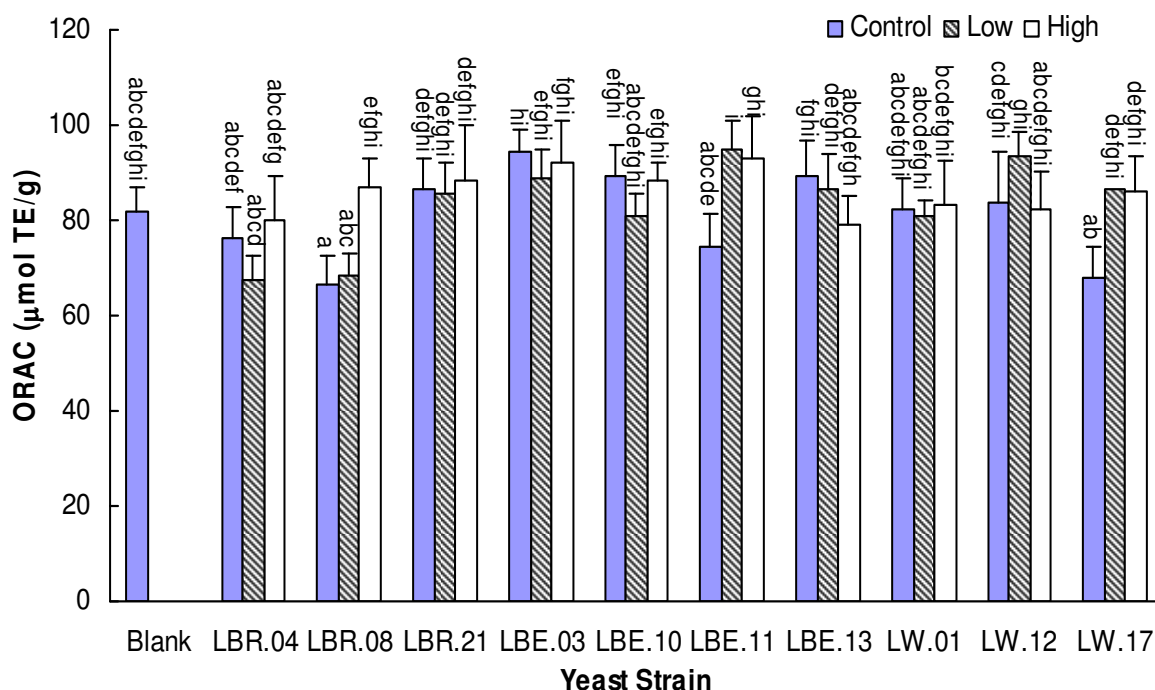
*ABTS<sup>•+</sup> Scavenging Capacity* – **Figure 2.2** compares the ABTS<sup>•+</sup> scavenging capacities of solid state yeast treated soft red bran with controls. In contrast with the minimal differences between controls and select treatments in TPC there were no significant differences between dose or yeast treatments.

*ORAC* – **Figure 2.3** demonstrates the ability of some yeast strains to significantly increase ORAC values in wheat bran when compared to their control. ORAC measures the radical scavenging capacity of the physiologically relevant

peroxyl radical, and may discontinue lipid peroxidation during the propagation step. All results are expressed as  $\mu\text{moles}$  of trolox equivalents per gram of wheat bran on a dry weight basis. Three yeasts significantly increased ORAC values including LBR.08, LBE.11, and LW.17. These data indicate the potential for using non-traditional bread yeasts in the treatment of bran and bran-based foods. It needs to be pointed out that as in TPC analysis, the control ORAC values for the controls are significantly different. More research is required to determine the contribution of yeasts on the ORAC assay.



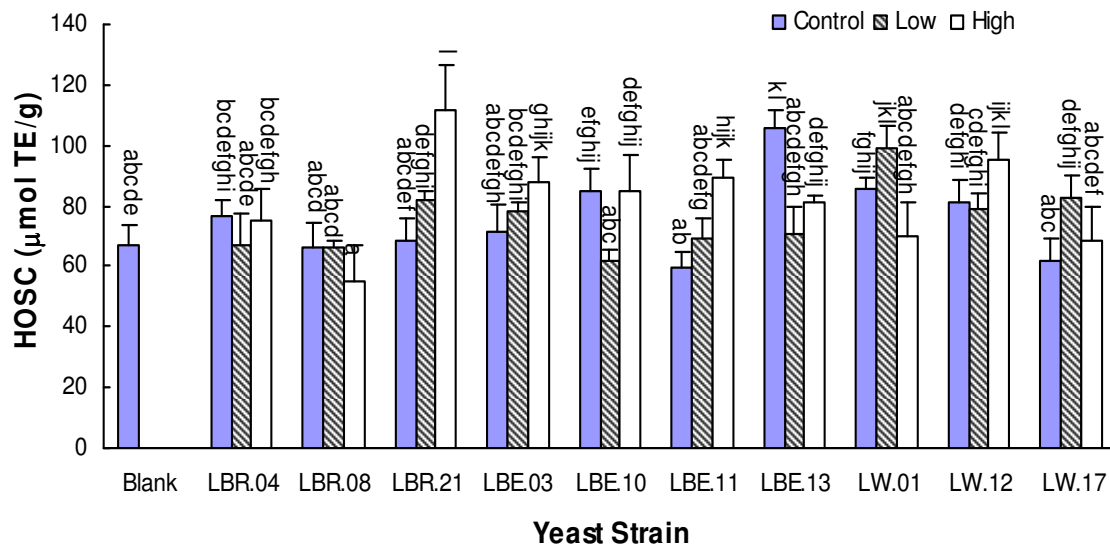
**Figure 2.2. ABTS<sup>+</sup> radical scavenging capacity of solid state yeast treated soft red wheat bran.** Results are expressed as Trolox equivalents (TE) per gram of solid state treated bran on a dry weight basis. Blank represents wheat bran run concurrently with sterile water. LBR.04, LBR.08, LBR.21, LBE.03, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 represent food grade yeasts from our laboratory yeast library. Control is inactivated yeasts; low and high were treated with  $5.6 \times 10^4$  and  $1.8 \times 10^5$  cells per g wheat bran, respectively at ambient temperature for 72 h. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported ( $n = 6$ ). Vertical bars represent the standard deviation of each data point. Values were not significantly different ( $P < 0.05$ ).



**Figure 2.3 Effect of solid state treatment on the oxygen radical absorbing capacities (ORAC) of soft red wheat bran.** Results are expressed as Trolox equivalents (TE) per gram of solid state treated bran on a dry weight basis. Blank represents wheat bran run concurrently with sterile water. LBR.04, LBR.08, LBR.21, LBE.03, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 represent food grade yeasts from our laboratory yeast library. Control is inactivated yeasts; low and high were treated with  $5.6 \times 10^4$  and  $1.8 \times 10^5$  cells per g wheat bran, respectively at ambient temperature for 72 h. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported (n = 6). Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

*HOSC* – **Figure 2.4** compares the ability of yeasts and their dose to enhance the hydroxyl radical scavenging capacity of wheat bran. When all yeasts and doses are compared only LBR.21 and LBE.11 demonstrate a dose dependent increase in  $\text{HO}^\bullet$  scavenging capacity. When each yeast strain is analyzed independently LBE.03 and LW.12 also show a statistically significant increase in hydroxyl radical scavenging as compared to their inactivated control. Remaining yeasts showed no

significant increases in hydroxyl radical scavenging activity versus their corresponding controls. It is important to mention the significant differences between the control values.

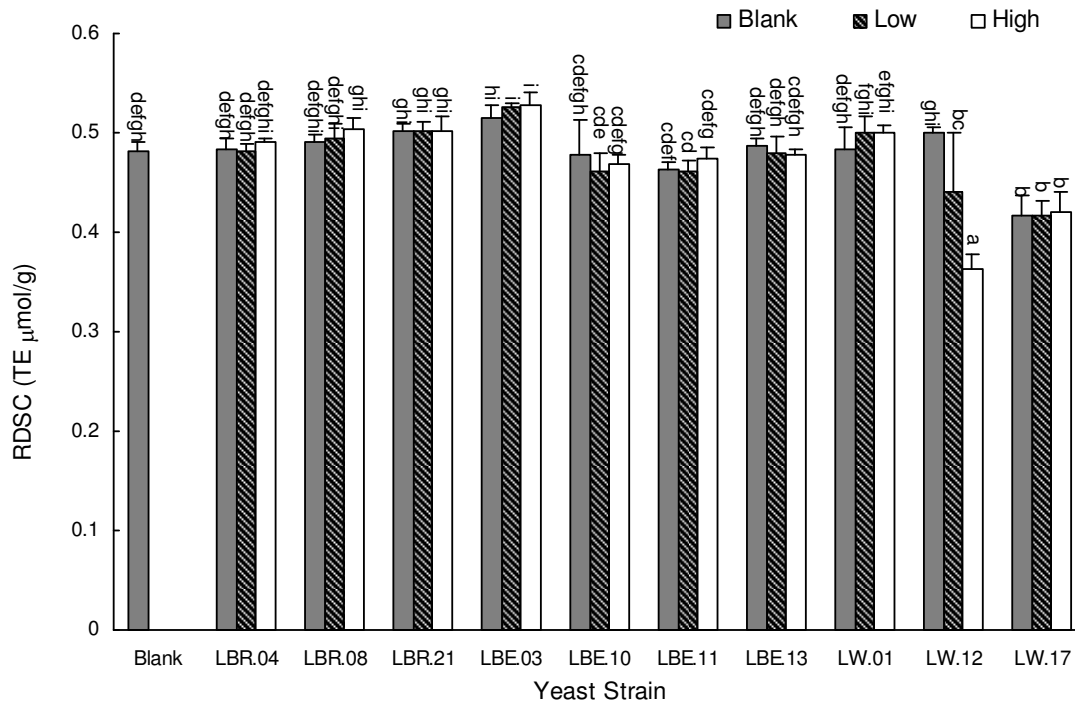


**Figure 2.4 Effect of solid state treatment on the hydroxyl radical scavenging capacities (HOSC) of soft red wheat bran.** Results are expressed as Trolox equivalents (TE) per gram of solid state treated bran on a dry weight basis. Blank represents wheat bran run concurrently with sterile water. LBR.04, LBR.08, LBR.21, LBE.03, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 represent food grade yeasts from our laboratory yeast library. Control is inactivated yeasts; low and high were treated with  $5.6 \times 10^4$  and  $1.8 \times 10^5$  cells per g wheat bran, respectively at ambient temperature for 72 h. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported ( $n = 6$ ). Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

**DPPH – Figure 2.5** compares the DPPH radical scavenging capacity of all yeasts at different doses and to that of the control and the blank. Results indicate that no yeasts were able to significantly increase DPPH scavenging capacity of soft red wheat bran. Similar to the other test results, the controls were significantly different,



indicating that the yeasts themselves might have different DPPH<sup>•</sup> scavenging components.



**Figure 2.5 Effect of solid state yeast treatment on the DPPH scavenging capacities of soft red wheat bran.** Results are expressed as Trolox equivalents (TE) per gram of solid state treated bran on a dry weight basis. Blank represents wheat bran run concurrently with sterile water. LBR.04, LBR.08, LBR.21, LBE.03, LBE.10, LBE.11, LBE.13, LW.01, LW.12, and LW.17 represent food grade yeasts from our laboratory yeast library. Control is inactivated yeasts; low and high were treated with  $5.6 \times 10^4$  and  $1.8 \times 10^5$  cells per g wheat bran, respectively at ambient temperature for 72 h. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported ( $n = 6$ ). Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

## Conclusion

These results show the challenge of using solid state yeast treatment to increase the potential antioxidant properties of soft red wheat bran. An important observation of this study is that solid state treatment may change the sensory properties of wheat bran, specifically strong scents, often fruity, were observed for

both dose levels. Slight genetic differences between *Saccharomyces cerevisiae* strains may be responsible for slight variances in fermentation rate, production of flavor chemicals, and nutrient preferences (Maloney & Foy 2003). This suggests that higher levels of yeasts may not be appropriate for all food-based applications, and that solid state yeast treatment warrants further research to develop bran-based foods with improved acceptability.

### Literature Cited

- Anson, N.M; Berg, R. V.D.; Havenaar, R.; Bast, A.; Haenen, G.R.M.M. Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *J. Agric. Food Chem.* **2008**. 56, 5589-5594.
- Bartalome, B.; Gomez-Cordoves, C.; Sancho, A.I.; Diez, N.; Ferreira, P.; Soliveri, J.; Copa-Patino, J.L. Growth and release of hydroxycinnamic acids from Brewer's spent grain by *Streptomyces avermitilis* CECT 3339. *Enzyme and Microbial Tech.* **2003**. 32, 140-144.
- Bayliak, M.; Semchyshyn, H.; Lushchak, V. Effect of hydrogen peroxide on antioxidant enzyme activities in *Saccharomyces cerevisiae* in strain-specific. *Biochemistry.* **2006**. 71(6), 1243-1252.
- Ejiofor, A.O.; Christi, Y.; Moo-Young, M. Culture of *Saccaromyces cerevisiae* on hydrolyzed waste cassava starch for production of baking-quality yeast. *Enzyme and Microbial Technology*, **1996**. 18, 519-515.
- Fang, Y.-Z.; Yang, S.; Wu, G. Free radicals, antioxidants, and nutrition. *Nutrition.* **2002**. 18, 872-879.
- Fernandez-Orozco, R.; Frias, J.; Munoz, R.; Zielinski, H.; Piskula, M.K.; Kozłowska, H.; Vidal-Valverde, C. Fermentation as a bio-process to obtain functional soybean flours. *J. Agric. Food Chem.* **2007**. 55, 8972-8979.
- Gutteridge, J.M.C.; Halliwell, B. Antioxidant protection and oxygen radical signaling. In: *Reactive Oxygen Species in Biological Systems, An Interdisciplinary Approach*; Gilbert, D.L.; Colton, C.A. Kluwer Academic/Plenum Publishers: New York, **1999**, 189-212.
- Lee, I.-H.; Hung, Y.-H.; Chou, C.-C. Solid-state fermentation with fungi to enhance the antioxidant activity, total phenolic content and anthocyanin contents of black bean. *International J. Food Micro.* **2008**. 121, 150-156.
- Liyana-Pathirana, C.M.; Shahidi, F. Antioxidant properties of commercial soft and hard winter wheat (*Triticum aestivum* L.) and their milling fractions. *J. Sci. Food & Ag.* **2006**. 86(3), 477-485.
- Mathew S.; Abraham T.E. Studies on the production of feruloyl esterase from cereal brans and sugar cane bagasse by microbial fermentation. *Enzyme and Microbial Technology*, **2005**. 36, 565-570.
- Miller, N.J., Rice-Evans, C.A. Spectrophotometric determination of antioxidant activity. *Redox Report.* **1996**. 2(3), 161-171.

- Moore, J.; Hao, J.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. Carotenoid, tocopherols, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**. 53(17), 6649-6657.
- Moore, J.; Cheng, Z.; Hao, J.; Guo, G.; Liu, J.-G.; Lin, C.; Yu, L. Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *J. Agric. Food Chem.* **2007**. 55, 10173-10182.
- Moore, J.; Cheng, Z.; Su, L.; Yu, L. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *J. Agric. Food Chem.* **2006a**. 54, 9032-9045.
- Moore, J.; Liu, J.-G.; Zhou, Z.; Yu, L. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J. Agric. Food Chem.* **2006b**. 54, 5313-5322.
- Oboh, G. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus spp* solid media fermentation techniques. *Electronic J. Biotech.* **2006**. 9(1), 46-49.
- Plessas, S.; Trantallidi, M.; Bekatorou, A.; Kanellaki, M.; Nigam, P.; Koutinas, A.A. Immobilization of *kefir* and *Lactobacillus casei* on brewery spent grains for use in sourdough wheat bread making. *Food Chem.* **2007**. doi: 10.1016/j.foodchem.2007.03.065.
- Shahidi, F. Functional foods: Their role in health promotion and disease prevention. *J. Food Sci.* **2004**. 69, 146-149.
- Slavin, J.L.; Jacobs, D.; Marquart, L. Grain processing and nutrition. *Crit. Rev. in Biotech.* **2001**. 21, 49-66.
- Stanner, S.A.; Hughers, J.; Kelly, C.N.M.; Buttriss, J. A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutr.* **2003**. 7(3), 407-422.
- USDA. Economic Research Services. (2007). Food Availability (Per Capita) Data System: Food Guide Pyramid. Accessed November 21, 2007. <http://www.ers.usda.gov/Data/FoodConsumption/FoodGuideIndex.htm#grain>.
- Wang, Y.-Y.D.; Fields, M.L. Feasibility of home fermentation to improve the amino acid balance of corn meal. *J. of Food Science.* **1978**. 43, 1104-1105.

- Wattanapitayakul, S.K.; Bauer, J.A. Oxidative pathways in cardiovascular disease roles, mechanisms, and therapeutic implications. *Pharmacology & Therapeutics*. **2001**. 89, 187-206.
- Wilcox, B.J.; Curb, J.D.; Rodriguez, B.L.; Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies. *Am J Cardiol*. **2008**. 101, 75D-86D.
- Yu, L.; Haley, S.; Perret, J.; Harris, M. Antioxidant properties of hard winter wheat extracts. *Food Chem*. **2002a**, 78, 457-461.
- Yu, L.; Haley, S.; Perret, L.; Harris, M.; Wilson, J.; Qian, M. Free radical scavenging properties of wheat extracts. *J. Agric Food Chem*. **2002b**. 50, 1619-1624.
- Yu, L.; Haley, S.; Perret, J.; Harris, M. Comparison of wheat flours grown at different locations for their antioxidant properties. *Food Chem*. **2004**, 86, 11-16.
- Yu, L.; Perret, J.; Harris, M.; Wilson, J.; Haley, S. Antioxidant properties of bran extracts from “Akron” wheat grown in different locations. *J Agric Food Chem*. **2003**. 56(24), 1566-1570.
- Yu, L.; Zhou, K. Antioxidant properties of bran extracts from 'Platte' wheat grown at different locations. *Food Chem*. **2004**, 90, 311-16.
- Zhou, K.; Laux, J. J.; Yu, L. Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. *J. Agric. Food Chem*. **2004a**, 52, 1118-1123.
- Zhou, K.; Su, L.; Yu, L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem*. **2004b**, 52, 6108-6114.
- Zhou, K.; Yu, L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem*. **2004c**, 52, 1112-1117.

### Chapter 3: Effects of Thermal Treatment, Fermentation, and Bran Particle Size on the Ethanol Extractable Antioxidant Properties of Whole-Wheat Pizza Crust

#### Abstract

This study investigated the impact of bran particle size, fermentation time, and baking time and temperature on the 100% ethanol extractable antioxidant capacities of whole wheat pizza crust. Trego and Lakin wheat varieties were used to prepare pizza crusts which were then analyzed for total phenolic content, ferulic acid content, and radical scavenging capacities against the peroxyl (ORAC), hydroxyl (HOSC), DPPH (RDSC) and cation ABTS radicals. Bran particle size did not significantly alter extractability of antioxidant properties. 18 hrs. of fermentation resulted in a 16.5 and 22.6% increase in RDSC and TPC values of 100% ethanol extracts of whole wheat pizza crusts. Three cooking combinations of 7 minutes at 400 °F, 14 minutes at 400 °F, and 7 minutes at 550 °F showed possible effects on available antioxidant properties in baked pizza crusts. Interestingly, the ratio of soluble free, soluble conjugated and bound ferulic acid in the pizza crusts was not significantly altered during thermal processing. Results suggest possible increases in ethanol extractable antioxidants in whole wheat pizza crusts by improving food preparation conditions.

## Introduction

Epidemiological studies show that a diet rich in foods containing antioxidants such as fruits, vegetables, and other plant derived foods decreases the risks of certain chronic diseases (Wattanapitayakul & Bauer 2001, Stanner et al. 2003, Wilcox et al. 2008). Oxidative defense mechanisms, protection from free radicals, is a primary mechanisms investigated to combat aging and age-related diseases (Wilcox et al. 2008). Recent in-vitro studies have demonstrated that antioxidants found in wheat are capable of scavenging free radicals such as the hydroxyl, peroxy, superoxide anion, DPPH, and cation ABTS radicals, chelate transition metals such as ionizing  $\text{Fe}^{2+}$ , and prevent lipid peroxidation (Yu et al. 2002, Zhou et al. 2004a, Zhou et al. 2004b, Zhou & Yu 2004, Adom et al. 2005, Moore et al. 2005).

Wheat (*Triticum spp.*) is an important commodity for U.S. consumers. It comprises 71 % of grain consumption and is one of the most commonly consumed grains globally (USHHS & USDA 2005). The most important factor in determining the antioxidant capacity of wheat products is the use of whole wheat rather than refined wheat flour (Halvorsen et al. 2006). The presence of bran in breads however has created sensory challenges for producers including loaf volume, darker crumb, and different flavor profiles (Lai et al. 1989, Hung et al 2007). One of the most widespread convenience foods worldwide, pizzas may make whole wheat pizza crust a good candidate to increase whole wheat consumption (Coppola et al 1998).

Food processing to develop higher quality whole wheat products may impact their antioxidant properties. Dough prepared with finely ground bran was found to be superior to dough prepared with conventionally ground bran. However, reduced

particle size reduces antioxidant stability during storage (Lai et al 1989, Cheng et al 2006a). Previous studies conducted in our laboratory showed that particle size had no effect on the 50% acetone antioxidant properties extractable in pizza crusts (Moore et al. 2008). A few studies have evaluated the affects of baking conditions on antioxidant levels in loaf breads, however limited data is available on the affects of processing on pizza dough properties and baked pizza crusts (Coppola et al. 1998, Lindenmeier et al. 2004, Halvorsen et al. 2006). Therefore, the objective of this study was to evaluate the effects of bran particle size, fermentation time, and thermal baking conditions on the ethanol extractable antioxidant properties and phenolic acid content of pizza dough and baked pizza crusts.

### Materials and Methods

#### **Chemicals & Reagents**

2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), flourescein (FL), FeCl<sub>3</sub>, and 2,2'-bipyridyl gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals, USA, Inc. (Richmond, VA). All other chemicals and solvents were of the highest commercial grade and used without further purification.



## **Wheat Flour Samples & Preparation**

Bran from hard winter wheat varieties, Trego and Lakin, were provided by Dr. Scott Haley of the Department of Soil and Crop Science, Colorado State University, Fort Collins, CO 80523. Samples had been grown in 2004 under typical agronomic practices for wheat production in eastern Colorado. Harvested grains were cleared of all non-grain debris present by seed cleaners and stored under ambient conditions. A Brabender Quadromat Junior experimental mill was used to grind grain samples and separate the bran fraction. A coffee grinder was then used to further grind the bran fraction to 20- and 80- mesh. Bran and non-bran fractions were then recombined in ratio to produce two varieties of whole wheat flour, each at two bran mesh sizes.

## **Dough & Pizza Crust Preparation**

Pizza dough was prepared by combining whole-wheat flour with water, honey, soybean oil, dry active yeast (Fleischmann's, Fenton, Missouri) and salt at w/w percentage ratios of 52.04, 33.24, 10.30, 2.34, 1.13, and 0.95 respectively. Dough was mixed using a KitchenAid stand mixer equipped with a dough hook on low speed for 4 minutes at ambient temperature. Dough was divided manually into 250 g pieces, rounded into balls, placed into stainless steel containers sprayed with Pam, and covered with plastic wrap. Fermentation was conducted at 4 °C for 0 (Control), 18, and 48 hours. Dough was tempered for 2 h prior to hand rolling into 12 inch pizza crusts. Crusts were hand transferred to aluminum pizza screens and baked at different time-temperature combinations in a conventional oven. Combinations included 7 minutes at 400 °F, 14 minutes at 400 °F, and 7 minutes at 550 °F.

Following 30 minutes cooling time, and crusts and dough samples were frozen, freeze dried, and ground to 40-mesh for extraction. Samples were stored in desiccators at ambient temperature in the dark until further analysis.

### **Sample Extraction Procedures**

Freeze dried pizza crusts and dough (Control) were extracted using 100% ethanol at a ratio of 1 gram material (dry weight basis) per 10 mL solvent, overnight. Samples were centrifuged and the supernatants collected and extracts used to determine total phenolic content, and ABTS cation, ORAC, and DPPH radical scavenging capacity. Extracts were stored under nitrogen in the dark at 20 °C until further analysis.

### **Total Phenolic Content**

Total phenolic content was measured using the Folin-Ciocalteu reagent according to a laboratory procedure (Yu et al 2002). The final reaction mixture contained 100 µL of sample extract, 500 µL of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate and 1.5 mL pure water. Absorbance at 765 nm was recorded after two hours incubation at ambient temperature. Gallic acid equivalence was calculated using a gallic acid standard curve. TPC analysis was conducted in duplicate with mean and standard deviation reported of triplicate thermal, mesh, or fermentation treatments combined (n = 6) .

### **Radical Cation ABTS•<sup>+</sup> Scavenging Capacity**

Sample extracts were tested against the ABTS•<sup>+</sup> cation radical generated according to laboratory protocol (Miller & Rice-Evans 1996, Zhou and Yu 2004). Briefly, an ABTS•<sup>+</sup> solution was prepared by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide 30 minutes at ambient temperature. The solution was then filtered and adjusted with phosphate buffer to an absorbance of 0.7 at a wavelength of 734 nm. A trolox standard is prepared with sample solvent with the blank consisting of 1mL phosphate buffer and 80 µL water blank. Likewise the final reaction mixture contained 1 mL of the ABTS•<sup>+</sup> solution, and 80 µL of the sample of trolox standard. Reaction mixtures were vortexed 30 seconds and a reading recorded at 1.5 minute. Data is reported as trolox equivalents (TE) per gram whole wheat pizza crust on a dry weight basis calculated using the trolox standard curve. Samples of the same thermal treatment, fermentation time, or mesh size were combined and reported as the mean (n = 6) and standard deviation.

### **Oxygen Radical Absorbance Capacity (ORAC) Assay**

ORAC was conducted on sample extracts using the fluorescent probe flourescein (FL) according to laboratory protocol (Huang et al 2002, Zhou and Yu 2004). Trolox standards and samples were prepared in the same solvent as the samples and AAPH and FL solutions were prepared with 75 mM phosphate buffer (pH 7.4). The final reaction mixture per well contained 225 µL of 81.63 µM FL solution, 30 µL blank (50% acetone), standard, or sample solution, and 25 µL 0.36 M AAPH solution. Therefore, each well contained a constant total volume of 280 µL.

Standards and samples were run in duplicate simultaneously utilizing a Victor<sup>2</sup> multilabel counter (PerkinElmer, Turku, Finland). Prior to AAPH addition, FL and antioxidant extracts were held at 20 minutes at 37 °C. The reaction mixture was held at 37 °C and fluorescence measurements taken every minute at excitation and emission wavelengths of 485 nm and 530 nm respectively for 1.5 hours. Results are expressed as trolox equivalents (TE) calculated using the relative area under the curve for the samples as compared to the trolox standard curve run simultaneously. Samples of the same thermal treatment, fermentation time, or mesh size were combined and reported as the mean (n = 6) and standard deviation.

#### **Radical DPPH Scavenging Capacity (RDSC).**

Free radical scavenging capacity of each sample was estimated following a previously reported procedure utilizing the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) (Yu et al. 2002), by the high-throughput radical scavenging capacity assay (RDSC) (Cheng et al. 2006a). The 200 µM DPPH<sup>•</sup> working solution was freshly prepared from a 1 mM DPPH<sup>•</sup> stock solution. Briefly, 100 µL of freshly made 200 µM DPPH<sup>•</sup> working solution was combined with 100 µL of solid state yeast treatment extract to start the radical-antioxidant reaction. On the same plate was also a blank of pure sample solvent, and control of solvent and 200 µM DPPH<sup>•</sup> working solution, and standards ranging from 7.8 to 250 mM Trolox. The absorbance at 517 nm was determined by Victor<sup>3</sup> multilabel plate reader over 40 minutes (Perkin-Elmer, Turku, Finland). The total area under the curve was calculated and used to determine the % DPPH radical quenched and RDSC value (TE mmol/g). Samples

were run in duplicate. Samples of the same thermal treatment, fermentation time, or mesh size were combined and reported as the mean (n = 6) and standard deviation.

### **Phenolic Acid Composition**

Baked pizza crusts were analyzed for their soluble free, soluble conjugated, and insoluble bound ferulic acid contents using a previously reported laboratory procedure (Moore et al. 2005). Acetone/methanol/water (7/7/6, v/v/v) was used to separate soluble and insoluble fractions. The supernatant containing soluble and conjugated free ferulic acid were separated further by acidifying the solution (pH 2) and extracting soluble free ferulic acid into ethyl ether/ethyl acetate (1/1, v/v). Soluble conjugated ferulic acid was then hydrolyzed with NaOH, re-acidifying the solution to a pH of 2, and the free ferulic acid was collected by ethyl ether/ethyl acetate (1/1, v/v) extraction. Bound ferulic acid in the solid residue was hydrolyzed with NaOH, supernatant acidified to a pH of 2 and the released ferulic acid was extracted with ethyl ether/ethyl acetate (1/1, v/v). The ethyl ether/ethyl acetate was evaporated using a nitrogen evaporator and extract re-dissolved in HPLC grade methanol. Samples were filtered using a 0.45  $\mu$ m membrane syringe filter and analyzed by HPLC using a Phenomenex C18 column (250 mm x 4.6 mm). A gradient elution program was used to separate the phenolic acids. Solvent A (acetic acid/H<sub>2</sub>O, 2:98, v/v) and solvent B (acetic acid/acetonitrile/ H<sub>2</sub>O, 2:30:68, v/v/v) were programmed from 10 – 100% B in 42 minutes with a flow rate of 1.5 mL/min (Zhou and Yu 2004). Identification and quantification of individual phenolic acids was accomplished by comparing the retention time and phenolic profile to that of known

concentrations of the pure phenolic compounds. HPLC analysis was conducted in duplicate and the value for the thermal treatment reported as the mean and the standard deviation.

### **Statistical Analysis**

All treatments (bran size, fermentation time, baking time and temperature) were conducted in triplicate. Data is reported as mean  $\pm$  SD of the combined treatments with individual extracts run in duplicate. One-way ANOVA and Tukey's HSD tests will be performed (Statistical Package for the Social Sciences (SPSS) for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to identify differences among means, with statistical significance declared at  $P \leq 0.05$ .

### **Results and Discussion**

#### ***Effects of Bran Mesh Size on the Antioxidant Properties of Baked Whole Wheat Pizza Crust.***

To evaluate the effects of bran particle size on 100% ethanol extractable pizza crust antioxidant properties, pizza crusts were prepared with two varieties (Lakin and Trego) of wheat, each having their bran ground to mesh sizes of 20 and 80. **Table 3.1** presents the results of antioxidant activity analysis of pizza crusts. These results indicate that reducing particle size slightly increased the ABTS•+ scavenging capacity for the Lakin variety, while slight decreases were observed in the Trego variety although neither change was significantly different. The significant increase

in antioxidant activity was observed in the ORAC test where reduced particles size resulted in an 11.8% increase for the Trego variety. Slight increases were also observed for DPPH• scavenging capacity although no significant differences were observed. Total phenolic contents followed the same trend as the ABTS•+ scavenging capacity assay.

**Table 3.1. Effect of Bran Mesh Size on Pizza Crust Antioxidant Properties for Two Hard Wheat Varieties\***

Wheat Variety	Mesh Size	ABTS ( $\mu\text{mol TE/ g pizza}$ crust dw)	ORAC ( $\mu\text{mol TE/g pizza}$ crust dw)	RDSC ( $\mu\text{mol TE/g pizza}$ crust dw)	TPC (mg GAE/g pizza crust dw)
Lakin	20	1.96 $\pm$ 0.08 a	1.83 $\pm$ 0.19 a	0.24 $\pm$ 0.03 a	0.34 $\pm$ 0.02 a
Lakin	80	2.46 $\pm$ 0.32 ab	2.21 $\pm$ 0.38 a	0.25 $\pm$ 0.03 a	0.31 $\pm$ 0.00 a
Trego	20	3.58 $\pm$ 0.46 b	2.63 $\pm$ 0.42 a	0.21 $\pm$ 0.01 a	0.32 $\pm$ 0.05 a
Trego	80	2.74 $\pm$ 0.62 ab	3.58 $\pm$ 0.16 b	0.23 $\pm$ 0.02 a	0.36 $\pm$ 0.01 a

\*Pizza crusts baked at 204 °C for 7 minutes. TE and GAE stand for trolox equivalents and gallic acid equivalents, respectively. All results are expressed on a per dry pizza dough or crust weight basis (dw). ABTS stands for ABTS•+ scavenging capacity; ORAC stands for oxygen radical absorbing capacity; RDSC stands for relative DPPH• scavenging capacity; TPC stands for total phenolic content. All sample extracts were analyzed in duplicate and reported mean values are the mean of the triplicate treatments  $\pm$  the standard deviation (n = 6). Values in the same test marked by the same letter are not significantly different ( $P < 0.05$ ).



Whole grain consumption is widely accepted as having components that maintain health and help resist age related diseases (Kantor et al. 2001, USHHS & USDA 2005, Hung et al. 2007, Mellen et al. 2008). Presence of bran and germ in wheat products to date is associated with decreased quality and sensory parameters such as lower loaf volume, darker crumb, and different flavor profiles (Lai et al. 1989, Hung et al 2007). Debranning flours completely may not be suitable for all applications either as debranning lowers the gluten index (Lijuan et al. 2007). Decreased particle size has been found to reduce antioxidant stability during storage, and antioxidant analysis for micro-ionized bran independently demonstrated that reduction of particle size may have the ability to increase antioxidant availability (Zhou et al. 2004b, Cheng et al. 2006b). A combination of grinding and presoaking bran using water and sodium stearyl lactylate was found to negate the effects of bran presence and maintain loaf volume (Lai et al 1989). Slight increases in antioxidant activity were observed in whole wheat pizza crust extracts prepared with 80-mesh bran flour. These results suggest that there were no significant decreases in antioxidant activity due to reduced particle size during pizza crust processing. Further analysis is required to determine the effects of bran particle size on the sensory attributes of non-loaf whole wheat products.

*Effects of Fermentation Time on the Antioxidant Properties of Whole Wheat  
Pizza Dough.*

Dough fermentation for the production of specialty bread products is an ancient technology. Most yeasts utilized in the baking industry are members of the

ascomycetous genus *Saccharomyces* with *Saccharomyces exiguous* and *Saccharomyces cerevisiae* used for sourdough, and modern baking yeasts, respectively (Maloney & Foy 2003). *Saccharomyces cerevisiae* strains are effective at producing carbon dioxide and ethanol from sugars and are used for modern baking, and beer and wine production (Maloney & Foy 2003). Carbon dioxide dissolves in the free water of dough and then expands in the gaseous form trapped by the gluten protein network, commonly known as dough rise (Poitrenaud 2004). The leavening process including leavening time is critical to maintain overall quality including 2-5 fold volume increase as well as acid production and rheological properties of baked products (Coppola et al. 1998). It is therefore of interest to understand how pizza dough fermentation may influence other minor components such as extractable antioxidant properties.

To evaluate the effects of fermentation time on 100% ethanol extractable pizza crust antioxidant properties, pizza crusts were prepared at a bran mesh size of 20, formed into 250 g balls and fermented for 0, 18, and 48 hrs. **Table 3.2** shows the antioxidant property of pizza dough samples. Results presented indicate that extended fermentation time of Lakin produced pizza doughs were either not significantly different from their control, or that antioxidant properties of the extracts were reduced. Similarly, Vitamin E and carotenoids losses were previously reported due to oxygenation during dough production, kneading, lipoxygenase activity, and heat destruction (Leenhardt et al. 2006). RDSC and TPC values from 100% ethanol extracts of whole wheat pizza crusts prepared with Trego wheat increased by 16.5% and 22.6% at 18 hrs. of fermentation over their controls, respectively. Previous study

of Trego wheat indicated that it has a unique capacity to inhibit lipid peroxidation in fish oil (Yu et al. 2002). More research is required to analyze how different distribution of phenolics in wheat bran may contribute to the different levels of antioxidant activity and their degradation or release during food preparation.

**Table 3.2 Effect of Fermentation Time on Pizza Crust Antioxidant Properties for Two Hard Wheat Varieties\***

Wheat Variety	Fermentation Time ( hrs.)	ABTS ( $\mu\text{mol TE/g}$ pizza crust dw)	ORAC ( $\mu\text{mol TE/g}$ pizza crust dw)	RDSC( $\mu\text{mol TE/g}$ pizza crust dw)	TPC (mg GAE/g pizza crust dw)
Lakin	0	$3.28 \pm 0.18$ b	$4.00 \pm 0.16$ ab	$0.30 \pm 0.01$ c	$0.36 \pm 0.02$ ab
Lakin	18	$3.08 \pm 0.06$ b	$4.02 \pm 0.18$ ab	$0.29 \pm 0.00$ bc	$0.34 \pm 0.03$ ab
Lakin	48	$3.17 \pm 0.04$ b	$3.30 \pm 0.46$ a	$0.20 \pm 0.01$ a	$0.35 \pm 0.03$ ab
Trego	0	$3.07 \pm 0.12$ b	$4.60 \pm 0.28$ bc	$0.26 \pm 0.02$ bc	$0.31 \pm 0.02$ a
Trego	18	$2.39 \pm 0.21$ a	$5.36 \pm 0.51$ c	$0.27 \pm 0.01$ bc	$0.38 \pm 0.02$ b
Trego	48	$2.46 \pm 0.00$ a	$4.73 \pm 0.71$ bc	$0.25 \pm 0.03$ ab	$0.37 \pm 0.00$ ab

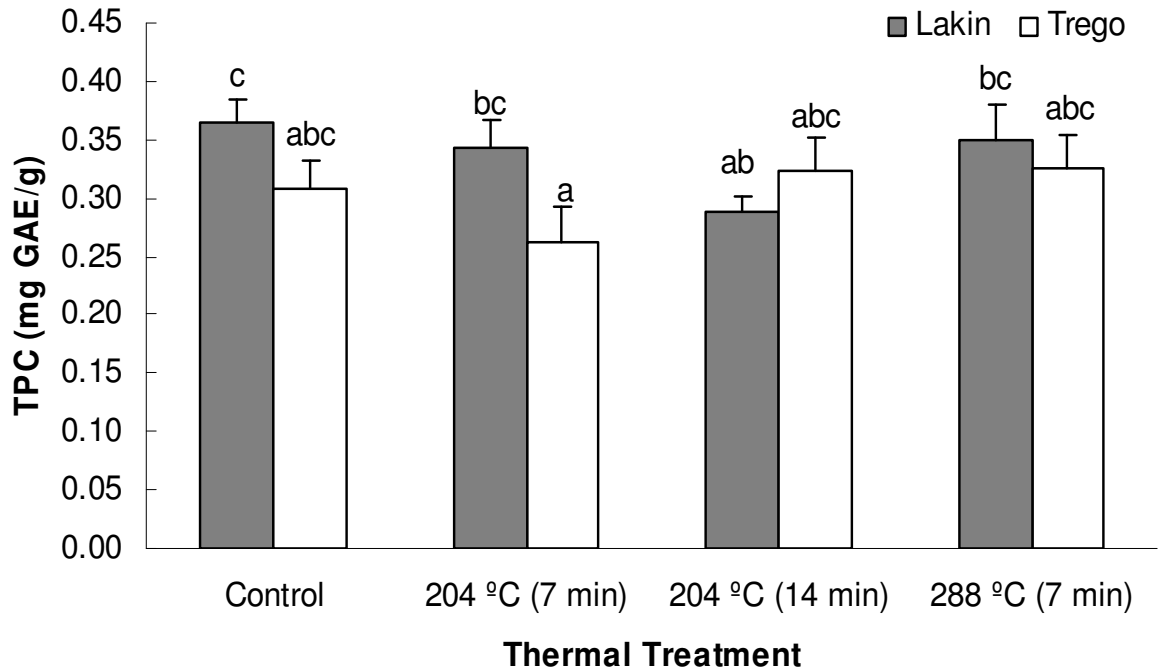
\*Pizza dough fermentation was conducted at 2 °C, covered in greased stainless steel containers. TE and GAE stand for trolox equivalents and gallic acid equivalents, respectively. All results are expressed on a per dry pizza dough weight basis (dw). ABTS stands for ABTS•+ scavenging capacity; ORAC stands for oxygen radical absorbing capacity; RDSC stands for relative DPPH• scavenging capacity; TPC stands for total phenolic content. All sample extracts were analyzed in duplicate and reported as the mean of the triplicate treatments  $\pm$  the standard deviation (n = 6). Values in the same column marked by the same letter are not significantly different ( $P < 0.05$ ).

*Effects of Baking Time and Temperature on the Antioxidant Properties of Baked Whole Wheat Pizza Crust.*

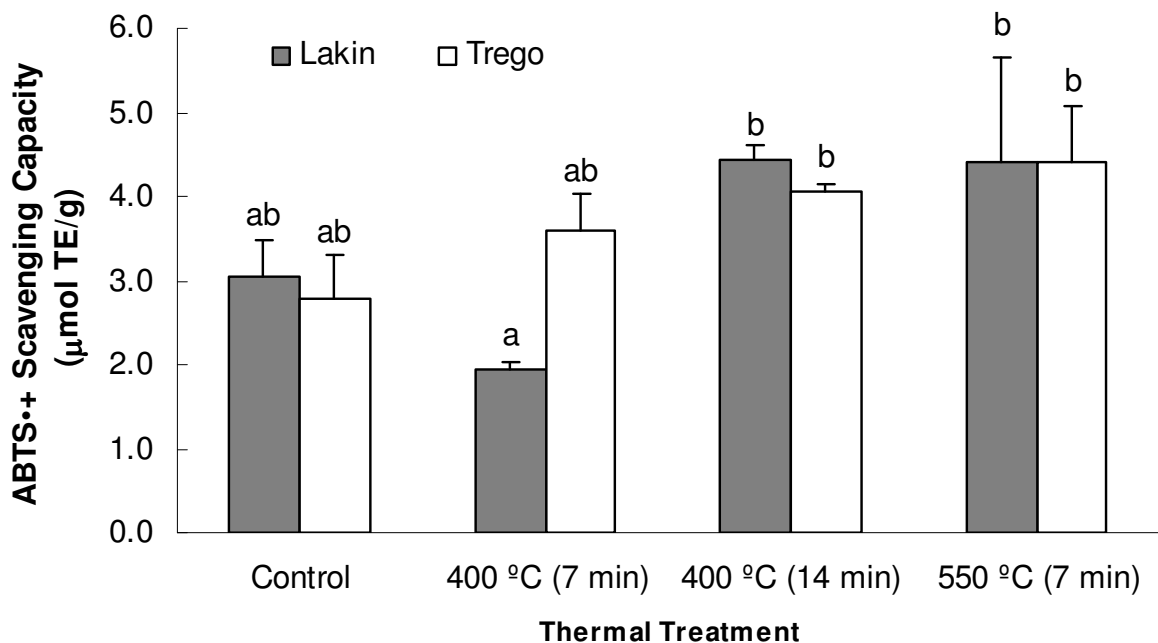
During food processing wheat antioxidant activity may be altered (Lindenmeier & Hofmann 2004, Leenhardt et al. 2006, Michalska et al. 2008). Increasing baking time and temperature increased antioxidant activity in the bread crust, but lowered antioxidant activity in the bread crumb portion (Lindenmeier & Hofman 2004). Toasting bread has also been found to increase antioxidant content 134-367% in bagels, French bread, white bread and whole wheat bread compared to their un-toasted counterparts (Halvorsen et al. 2006). Maillard reaction products have received significant attention as they may be potential carcinogens, however study of bakery product fractions also indicate that areas subject to the browning reaction have higher antioxidative potential in contrast to their corresponding inner counterparts (Lindenmeier & Hofman 2004). It is therefore of interest to analyze the impact of thermal treatment on the existing antioxidant compounds in wheat, as well as the impact of pizza crust as a baked item with a large surface area.

*Total Phenolic Content (TPC)* – **Figure 3.1** shows that of the baking conditions evaluated Lakin wheat pizza crust total phenolic content (TPC) was more stable at shorter heat exposure while TPC of Trego wheat pizza crusts had slightly higher although neither increased time nor temperature combinations significantly altered TPC content. TPC values in this study of whole wheat pizza crusts are comparable to those of 100% ethanol extracts of Ankor wheat grain (Cheng et al. 2006b). Therefore, including whole wheat and wheat bran into foods supports the

notion that wheat may serve as a good source for phenolic compounds in functional foods as a major constituent of food formulas.



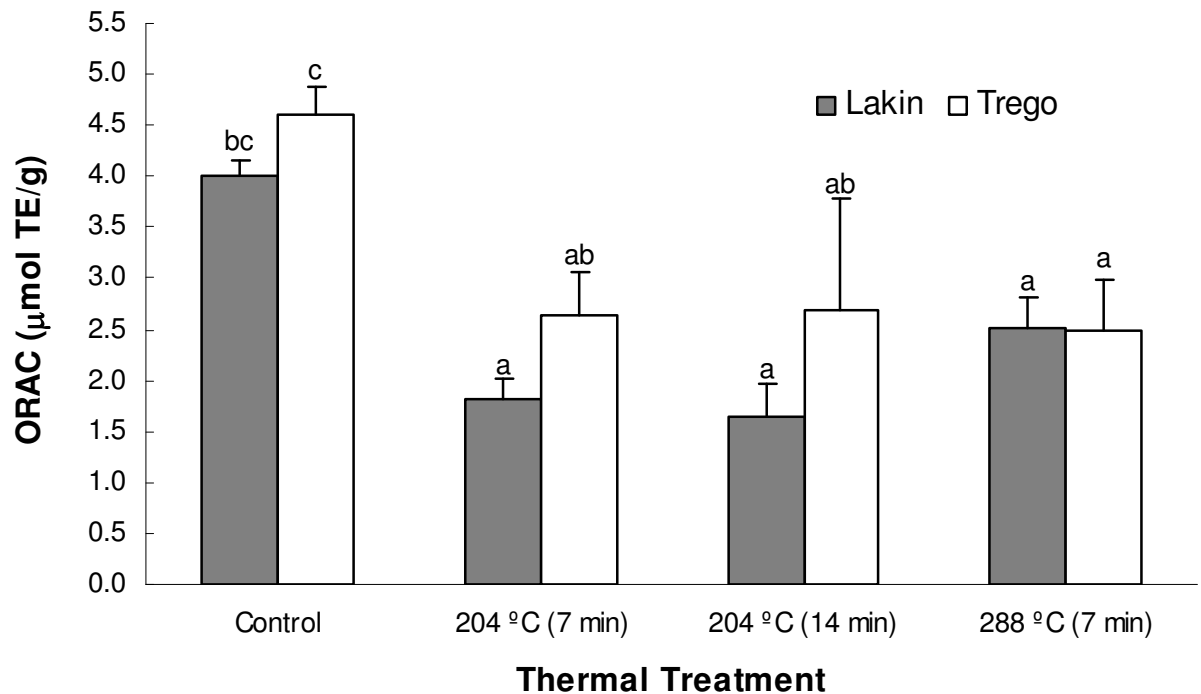
**Figure 3.1 Effect of thermal treatment on the total phenolic content of 100% ethanol extracts of whole wheat pizza crust.** Control represents dough samples not baked. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. Results are expressed as gallic acid equivalents (GAE) per g of sample on a dry weight basis. All measurements were conducted in duplicate with the mean value of the triplicate yeast dose treatment combined reported. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).



**Figure 3.2 Effect of thermal treatment on scavenge capacity of the cation ABTS•+ radical content of 100% ethanol extracts of whole wheat pizza crust.** Results are expressed as Trolox equivalents (TE) per gram of solid state treated bran on a dry weight basis. Control represents dough samples not baked. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. All measurements were conducted in duplicate with triplicate samples of the yeast/dose combined. Mean (n = 6) values are reported and standard deviations indicated by the vertical bars over each data point. No significant differences were detected ( $P < 0.05$ ).

*Radical Cation ABTS•+ Scavenging Capacity* – **Figure 3.2** compares the ABTS•+ scavenging capacities of baked whole wheat pizza crusts. Slight increases were observed for all baked pizza crusts compared to their dough controls with the exception of minimally processed Lakin pizza crust. Lakin and Trego based pizza crusts showed increased ABTS•+ radical scavenging by 45.3 and 58.8% for extended baking time and 45.9 and 45.9% for increased temperature, respectively. ABTS•+ radical scavenging increases were between 42 and 47% for previously reported 50% acetone extracts of baked whole wheat pizza crusts (Moore et al. 2008). ABTS•+

radical scavenging capacity of wheat bran was previously shown to be temperature sensitive during wheat storage (Cheng et al. 2006b). These data suggest that whole wheat foods as opposed to wheat bran independently may resist the loss of natural antioxidants under elevated temperatures.



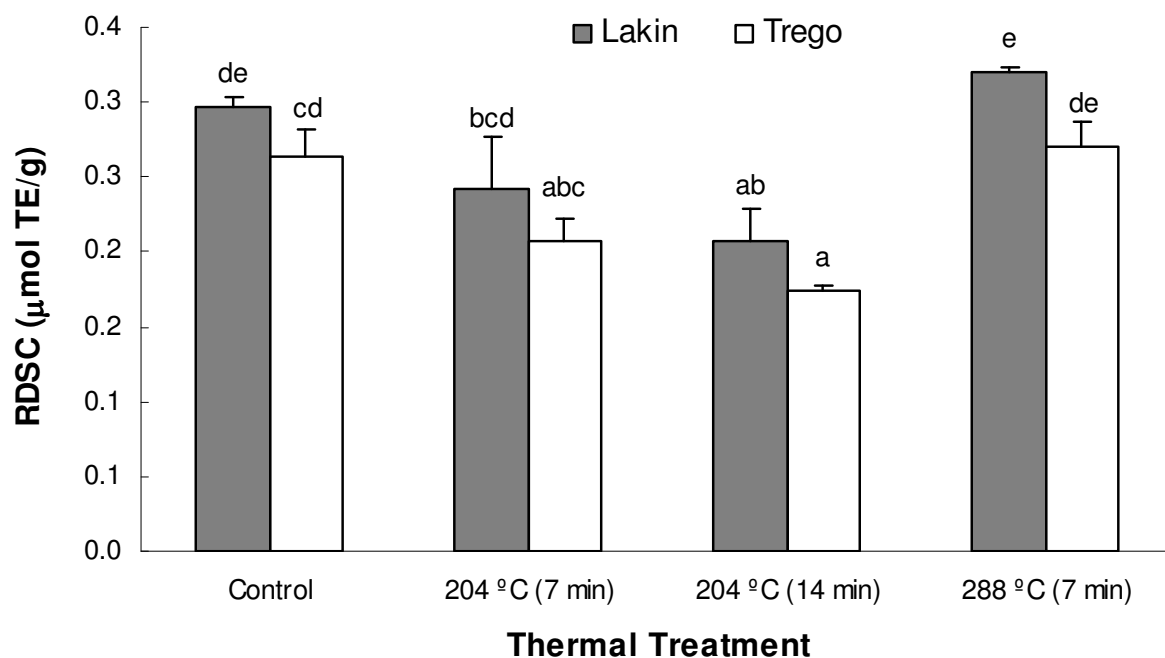
**Figure 3.3 Effect of baking time and temperature on the oxygen radical absorbing capacities of whole wheat pizza crusts.** Results are expressed as Trolox equivalents (TE) per gram of pizza crust on a dry weight basis. Control represents dough samples not baked. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. Measurements were conducted in duplicate with triplicate samples of the yeast/dose combined. Mean (n = 6) values are reported and standard deviations indicated by the vertical bars over each data point. No significant differences were detected ( $P < 0.05$ ).

*Oxygen Radical Absorbance Capacity (ORAC)* – **Figure 3.3** shows that time and temperature combinations had no significant impact on ORAC values however all baked crusts were significantly lower than their dough controls. ORAC values for baked pizza crusts reported here are significantly higher than those reported for whole



wheat bread on a per serving basis (Wu et al. 2004). Taking loss of ORAC activity during processing into account, whole wheat pizza crust still has ORAC values which may have a positive effect on overall health when consumed as part of a healthy diet.

*Radical DPPH Scavenging Capacity (DPPH)* – **Figure 3.4** shows the DPPH radical scavenging capacity of pizza crusts baked under specified thermal treatments and their control. Results indicate that increasing temperature increased RDSC value by 31.8 and 29.8% for Lakin and Trego based pizza crusts, respectively. In contrast, increasing baking time decreased RDSC values by 14.4 and 16.6% for Lakin and Trego based pizza crusts, respectively. While increased temperature did not significantly increase RDAC values to their respective controls, values did not decrease indicating that shorter exposure to high temperatures may be suitable means of food processing to maintain DPPH scavenging capacity.

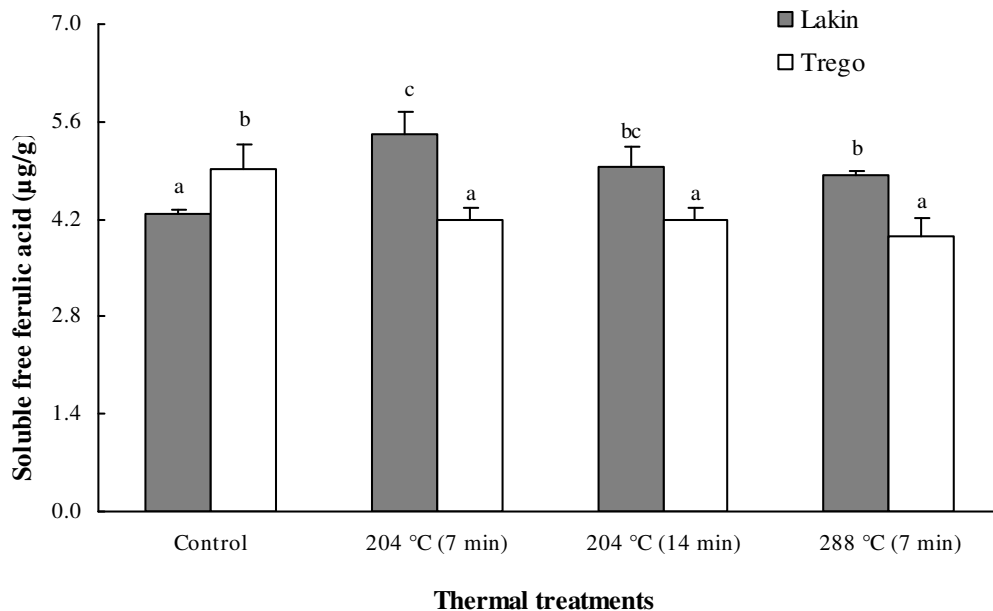


**Figure 3.4: Effect of baking time and temperature on the DPPH scavenging capacities of whole wheat pizza crusts.** Results are expressed as Trolox equivalents (TE) per gram of pizza crust on a dry weight basis. Control represents dough samples not baked. Treatments were baked in a conventional oven for their respective times and temperatures. Measurements were conducted in duplicate with triplicate samples of the yeast/dose combined. Mean ( $n = 6$ ) values are reported and standard deviations indicated by the vertical bars over each data point. No significant differences were detected ( $P < 0.05$ ).

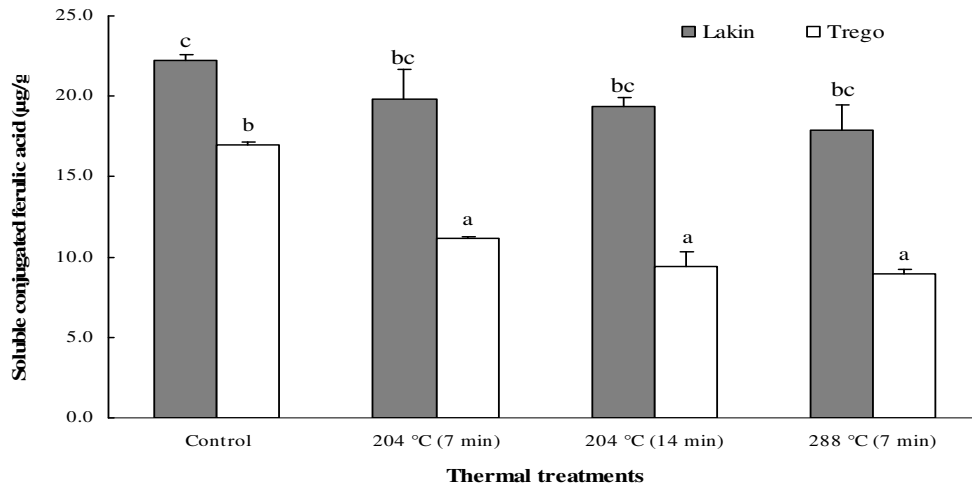
*Ferulic Acid Content of Baked Pizza Crusts* – **Figures 3.5- 3.7** contain the quantity of soluble free, soluble conjugated and bound ferulic acid from whole wheat pizza crusts prepared under selected baking conditions. Significant increases in soluble free ferulic acid of 26.6% were demonstrated when pizza crusts were baked at 204 °C for 7 minutes as compared with their dough control. It needs to be pointed out that pizza crusts prepared with the Trego wheat variety had a significant decrease in soluble free ferulic acid; however neither extending baking time nor increasing

baking temperature demonstrated further reduction over basic baking conditions. Soluble conjugated ferulic acid was significantly decreased for all baking time and temperature combinations of pizza crusts prepared with the Trego variety but not for Lakin (**Fig 3.6**). Insoluble bound ferulic acid remained unaffected by thermal processing (**Fig. 3.7**). It is interesting to note that baked pizza crusts prepared with the Lakin variety had significantly more soluble conjugated ferulic acid, while baked pizza crusts prepared with the Trego variety had significantly more bound ferulic acid. Previous studies of the Trego variety have indicated it's unique potential to inhibit lipid peroxidation, which was not explained by its radical scavenging and chelating activities (Yu et al 2002). These data indicate that wheat variety differences in antioxidant activity may be carried over into whole wheat functional foods.

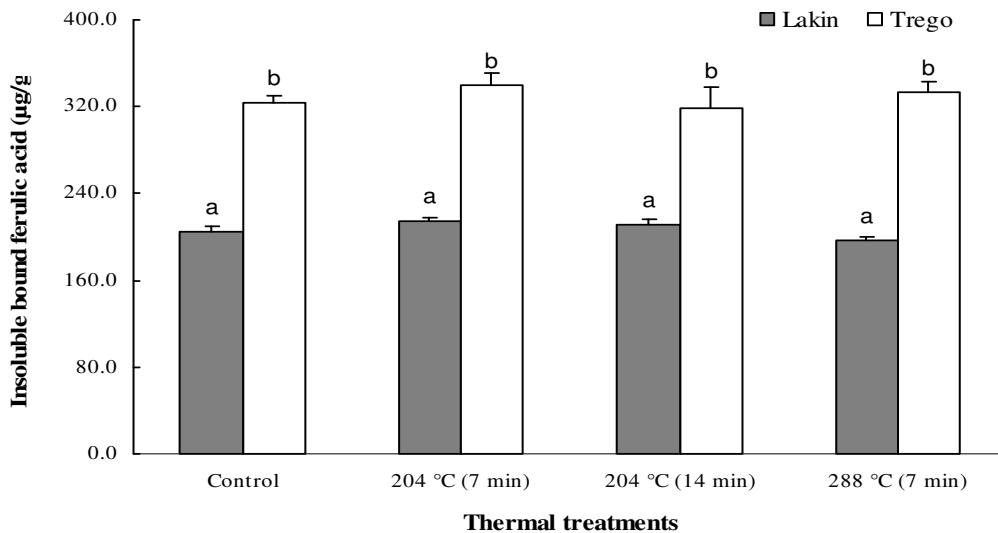
**Figure 3.5: Effect of baking time and temperature on soluble free ferulic acid of whole wheat pizza crusts\***



**Figure 3.6: Effect of baking time and temperature on soluble conjugated ferulic acid of whole wheat pizza crusts\***



**Figure 3.7: Effect of baking time and temperature on insoluble bound ferulic acid of whole wheat pizza crusts\***



**Figures 3.5 – 3.7\*** Results are expressed as Trolox equivalents (TE) per gram of pizza crust on a dry weight basis. Control represents dough samples not baked. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. Measurements were conducted in duplicate with triplicate samples of the yeast/dose combined. Mean ( $n = 6$ ) values are reported and standard deviations indicated by the vertical bars over each data point. No significant differences were detected ( $P < 0.05$ ).

### Conclusion

Results from this study indicate that whole wheat pizza crusts maintain significant antioxidant activity following food processing. The pizza crusts prepared from two varieties of wheat, Lakin and Trego, differed significantly in soluble free and bound ferulic acid indicating that significant differences in antioxidant activities of wheat variety may be carried over in finished products. Reducing of bran particle size may not result in the deterioration of antioxidant activity in the pizza crust model suggesting that reduced particle size may be one means of improving whole wheat product sensory products, while controlling for the loss of natural antioxidants. Antioxidant activities were also generally stable through extended fermentation times indicating the possibility to develop functional wheat based foods with sufficient rise and unique flavor character profiles. Further studies are required to understand the thermal processing effects on whole wheat food systems and to optimize conditions for desired sensory and health beneficial properties.

### Literature Cited

- Adom, K.K.; Sorrells, M.E.; Liu, R.H. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *J. Agric. Food Chem.* **2005**, 53, 2297-2306.
- Cheng, Z.; Moore, J.; Yu, L. A high-throughput relative DPPH radical scavenging capacity (RDSC) assay. *J. Sci. Food Agric.* **2006a**, 54, 7529-7436.
- Cheng, Z.; Su, L.; Moore, J.; Zhou, K.; Luther, M.; Yin, J.; Yu, L. Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *J. Agric. Food Chem.* **2006b**, 54, 5623-5629.
- Coppola, S.; Pepe, O.; Mauriello, G. Effect of leavening microflora on pizza dough properties. *J. Applied Micro.* **1998**, 85, 891-897.
- Halvorsen, B.L.; Carlsen, M.H.; Phillips, K.M.; Bohn, S.K.; Holte, K.; Jacobs, D.R. Jr.; Blomhoff, R. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *Amer. J. Clin. Nutr.* **2006**, 84, 95-135.
- Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J.A.; Deemer, E.K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated  $\beta$ -cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**, 50, 1815-1821.
- Hung, P.V.; Maeda, T.; Morita, N. Dough and bread qualities of flours with whole waxy wheat flour substitution. *Food Research Inter.* **2007**, 40, 273-279.
- Kantor, L.S.; Variyam, J.N.; Allshouse, J.E.; Putnam, J.J.; Lin, B.-H. Choose a variety of grains daily, especially whole grains: A challenge for consumers. *J. Nutr.* **2001**, 131, 473S-486S.
- Lai, C.S.; Hoskeney, R.C.; Davis, A.B. Effects of wheat bran in breadmaking. *Cereal Chem.* **1989**, 66(3), 217-219.
- Leenhardt, F.; Lyan, B.; Rock, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesy, C. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *J. Agric. Food Chem.* **2006**, 54, 1710-1715.
- Lijuan, S.; Guiying, Z.; Guoan, Z.; Zaigui, L. Effects of different milling methods on flour quality and performance in steamed breadmaking. *J. Cereal Sci.* **2007**, 45, 18-23.

- Lindenmeier, M. & Hofmann, T. Influence of baking conditions and precursor supplementation on the amounts of the antioxidant Pronyl-L-lysine in bakery products. *J. Agric Food Chem.* **2004.** 52, 350-354.
- Maloney, D.H.; Foy, J.J. Yeast Fermentations. In “Handbook of Dough Fermentations”. Kulp, K.; Lorenz, L. (Eds). Food Science & Technology. New York, NY. **2003.** 43-62.
- Mellen, P.B.; Walsh, T.F.; Herrington, D.M. Whole grain intake and cardiovascular disease: A meta-analysis. *Nutrition, Metabolism & Cardiovascular Disease.* **2008.** 18, 283-290.
- Michalska, A.; Amigo-Benavent, M.; Zielinski, H.; Dolores del Castillo, M. Effect of bread making on fermentation of Maillard reaction products contributing to the overall antioxidant activity of rye bread. *J. Cereal Sci.* **2008.** 48, 123-132.
- Miller, N.J., Rice-Evans, C.A. Spectrophotometric determination of antioxidant activity. *Redox Report.* **1996.** 2(3), 161-171.
- Moore, J.; Hao, J.; Zhou, K.; Luther, M.; Costa, J.; Yu L. Carotenoid, tocopherols, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005.** 53(17), 6649-6657.
- Moore, J.; Luther, M.; Cheng, Z.; Yu, L. Effects of baking conditions, dough fermentation, and bran particle size on antioxidant properties of whole-wheat pizza crust. *J. Agric Food Chem.* **2008.** In submission.
- Poitrenaud, B. Baker's yeast. In “Handbook of Food and Beverage Fermentation Technology.” Hui, Y.H.; Hansen, A.S.; Josephsen, J.; Nip, W.-K.; Stanfield, P.S.; Toldra, F. (Eds). Food Science & Technology. New York, NY. **2004.** 695-719.
- Stanner, S.A.; Huijbers, J.; Kelly, C.N.M.; Buttriss, J. A review of the epidemiological evidence for the ‘antioxidant hypothesis’. *Public Health Nutr.* **2003.** 7(3), 407-422.
- U.S. Departments of Health and Human Services and U.S. Department of Agriculture. **2005.** Dietary Guidelines for Americans, 2005. 6<sup>th</sup>. Edition, Washington, DC: U.S. Government Printing Office.
- Wattanapitayakul, S.K.; Bauer, J.A. Oxidative pathways in cardiovascular disease roles, mechanisms, and therapeutic implications. *Pharmacology & Therapeutics.* **2001.** 89, 187-206.

- Wilcox, B.J.; Curb, J.D.; Rodriguez, B.L.; Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies. *Am J Cardiol.* **2008**, 101, 75D-86D.
- Wu, X.; Beecher, G.R.; Holden, J.M.; Haytowitz, D.B.; Gebhardt, S.E.; Prior, R.L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **2004**, 52, 4026-4037.
- Yu, L.; Haley, S.; Perret, J.; Harris, M. Antioxidant properties of hard winter wheat extracts. *Food Chem.* **2002**, 78, 457-461.
- Zhou, K.; Su, L.; Yu, L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004a**, 52, 6108-6114.
- Zhou, K.; Laux, J. J.; Yu, L. Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. *J. Agric. Food Chem.* **2004b**, 52, 1118-1123.
- Zhou, K.; Yu, L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem.* **2004**, 52, 1112-1117.



## Chapter 4: In-vitro Bioavailability of Wheat Antioxidants in Baked Pizza Crust.

### Abstract

This study evaluates the effect of baking time and temperature on the in-vitro bioavailability of wheat antioxidants in baked pizza crusts. Pizza crusts were prepared under three baking conditions of 7 minutes at 400 °F, 14 minutes at 400 °F, and 7 minutes at 550 °F. After in-vitro digestion, the supernatant was separated from the solid residue. Acetone was added to the supernatant for clarification and re-centrifuged resulting in 37.5% acetone supernatant solutions. The supernatant solutions were then analyzed for total phenolic content (TPC), and radical scavenging activity against peroxy and hydroxyl anion and the ABTS<sup>•+</sup> cation radicals. The available total phenolic content ranged between 2.8 and 5.2 gallic acid equivalents (GAE) for dough and thermal treatment of 288 °C for 7 minutes, respectively. Likewise the ABTS<sup>•+</sup> scavenging capacity increased with thermal treatment with a range of 3.6 – 4.5 µmoles trolox equivalents (TE) per gram pizza crust, oxygen radical absorbance capacity had a range of 112.2 – 190.0 µmoles TE/g, and hydroxyl radical scavenging had a range of 77.4 – 174.0 µmoles TE/g. Pizza crust samples prepared under higher temperatures or longer baking times had higher in-vitro bioavailable TPC and ABTS<sup>•+</sup> scavenging capacity. In-vitro bioavailability of ORAC and HOSC antioxidant capacity assays also suggest that thermally treated samples might have greater available antioxidant activities than that of their controls.

Understanding the bioavailability of antioxidants from the food matrix may assist in the development of physiologically active functional foods.

### Introduction

It is now recommended that Americans consume at least 3 or more once-equivalence of whole grains per day in order to reduce the risk of several chronic diseases and assist with weight management (USDA 2005). Wheat is a prominent ingredient for the Western diet with intake of approximately 133 pounds per person annually in the United States (USDA-ERS 2006). Epidemiological studies provide support that whole foods, such as those prepared with whole grains, have a greater effect against a broad range of diseases than isolated components (Slavin, 2003).

Antioxidant research on Swiss red wheat has determined that the aleurone portion of the wheat kernel has the highest level of antioxidants followed by bran and whole grain (Zhou et al. 2004). Multiple wheat varieties contain antioxidants with the ability to chelate  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ , and quench anion, neutral, and cation free radicals (Zhou & Yu 2004, Zhou et al. 2004, Yu & Zhou 2004, and Zhou et al. 2005). This antioxidant activity may be due to the presence of phenolic compounds such as flavanoids, phenolic acids, and catechins as found in the bran of whole grains (Adom et al. 2005, Ross et al. 2004). Phenolic acids are generally found in the bound form and it is unclear what portion of these phenolics is available for absorption in the digestive tract (Kern et al. 2003, Zhou et al. 2004, Price et al. 2008).

Post harvest treatment and food processing may have an effect on the available antioxidants from wheat. Storing wheat as a whole grain for long-term storage may help prevent the loss of natural antioxidants (Cheng et al. 2006). For immediate use, reduction of particle size increases bioavailability of phenolics (Cheng et al. 2006). Post harvest treatment of wheat bran with enzymes and yeasts have demonstrated the potential for solid state treatment to increase the proportion of soluble free antioxidants from their native bound form (Moore et al. 2006, Moore et al. 2007). Not all compounds and types of processing increase antioxidant activity as demonstrated by Leenhardt et al. who found that carotenoids levels decreased during kneading, fermentation, and baking (Leenhardt et al. 2006). Research conducted previously in our laboratory has shown that increasing baking time or baking temperature increases the total phenolic content and antioxidant activity of whole-wheat pizza crusts (Moore 2008).

Limited studies have been conducted to measure the *in vivo* impact of consuming whole wheat products or wheat bran. *In vitro* research has shown that the total phenolic content (TPC) and total antioxidant activity (TAA) of wheat extracts was increased after simulated pH digested conditions (Liyanapathirana & Shadidi 2004). Following high bran cereal consumption limited ferulic and sinapic acids were taken up by humans and authors concluded that in this food model, bound phenolic acids were not absorbed (Kern et al. 2003). Higher plasma uptake of phenolics was detected in untreated wheat bran indicates that wheat bran phenolics are relatively well absorbed with plasma total phenolic increases comparable to red wine and spinach (Price et al. 2008). These two reports utilizing 6 and 17 human

subjects are the only studies we know of analyzing the absorption of phenolics from wheat by humans.

A prominent item in the western-diet, this study analyzes the effects of thermal treatment on in-vitro bioavailability of antioxidants from whole wheat pizza crusts. In-vitro bioavailability of antioxidants was then measured by analyzing supernatants for their total phenolic content, and radical scavenging activity against the peroxyl, hydroxyl, and ABTS•+ radicals.

### Materials and Methods

Digestive fluid chemicals; potassium chloride, magnesium chloride, D-glucuronic acid, D(+)-glucosamine hydrochloride, bile from bovine and ovine, and pepsin as well as reagents mixed tocopherols, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), disodium ethylenediaminetetracetate, pancreatin, and 2,2'-bipyridyl gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). Urea, ammonium chloride, sodium phosphate, sodium chloride, calcium chloride, and potassium thiocyanate were purchased from Fisher Scientific and were used without further purification. Sodium bicarbonate and sodium sulfate were purchased through J.T. Baker (Phillipsburg, NJ), D(+)-glucose and uric acid were purchased through Acros (Morris Plains, NJ). Folin-Ciocalteu reagent was freshly prepared in the laboratory. All other chemicals and solvents were of the highest commercial grade and used without further purification.

### **Preparation of pizza crust samples**

Pizza crusts were prepared from whole-grain flours of Trego and Lakin hard winter wheat varieties provided by Dr. Scott Haley in the Department of Soil and Crop Science, Colorado State University, Fort Collins, CO 80523. Briefly, the bran fraction of the grain was separated and ground to a 20-mesh using a coffee grinder and recombined to create whole-grain flour. Whole-grain flour, water, honey, soybean oil, dry active yeast (Fleischmann's Fenton, Missouri), and salt were then combined on a w/w ratio of 52.04, 33.24, 10.30, 2.34, 1.12 and 0.95 grams respectively. Dough was then mixed with a Kitchen-Aid stand mixer for 1 minute on low speed, followed by 3 minutes of hand kneading. 250 g pieces were then rounded and placed onto greased stainless steel containers and allowed to ferment 18 hrs. at 4 °C. Dough was incubated at room temperature for 2 hrs. prior to hand rolling into 12 inch crusts. Crusts were baked on greased aluminum pizza screens in a conventional oven at baking combinations of; 7 minutes at 208 °C, 14 minutes at 208 °C, and 7 minutes at 288 °C. After cooling for 30 minutes baked samples as well as the control (unbaked fermented dough), were frozen, freeze-dried, and ground to a 40-mesh.

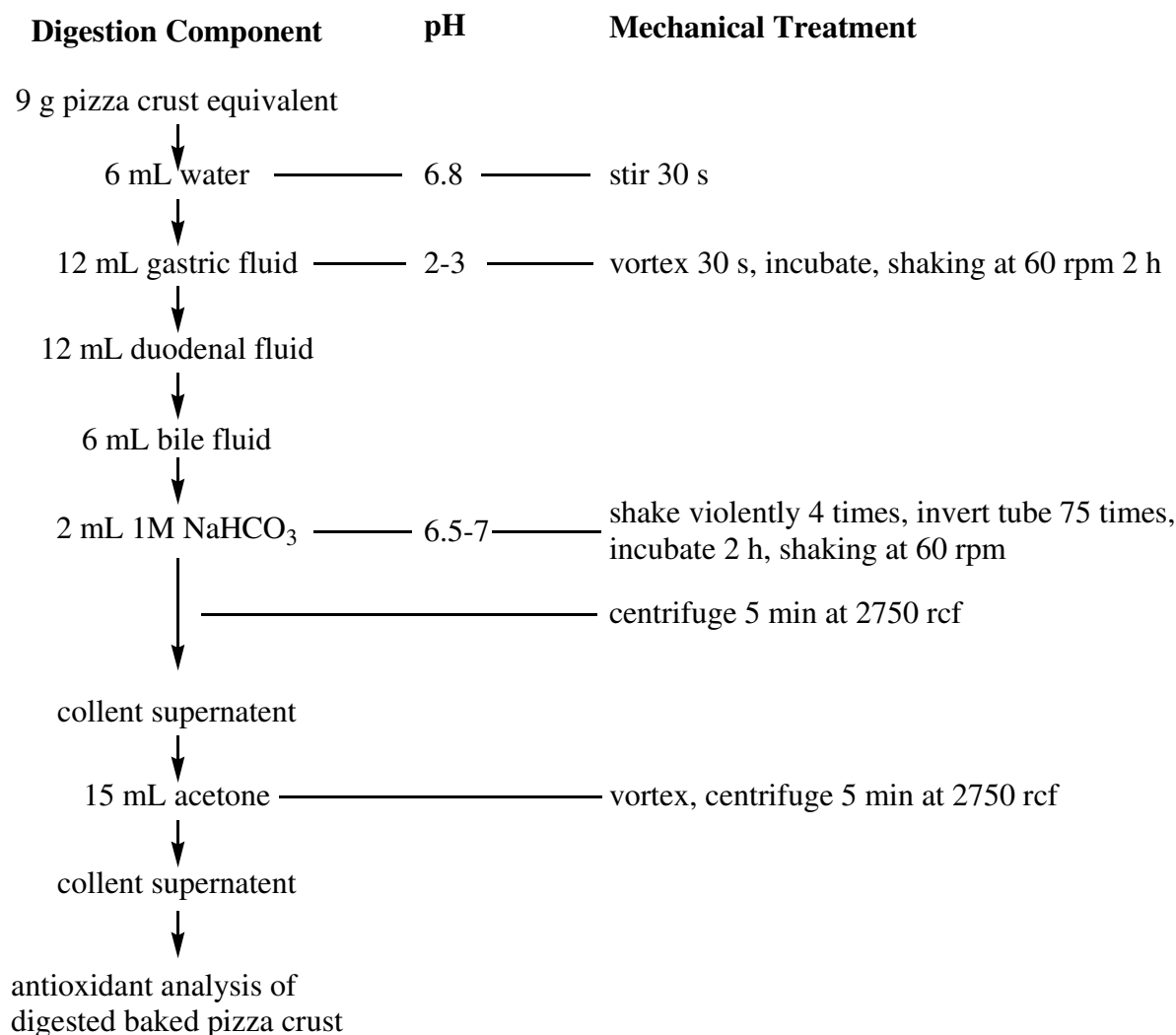
### **In-vitro Digestion**

The purpose of the in-vitro digestion model is to simulate the digestion process in the gastrointestinal tract by replicating physiological conditions. This model is based upon the models introduced by Oomen et al. (2003), Versantvoort et al. (2005), and the United States Pharmacopeia specifications for digestive fluids.

Digestive fluids were prepared according to fed-state conditions (**Table 4.1**). Nine grams of pizza crust equivalent was used per sample by re-hydration of the sample to 25% moisture, the average moisture content of baked pizza crust. Duplicate samples of 40-mesh, freeze dried pizza crust were weighed into 50 mL disposable test tubes. **Figure 4.1** depicts the manner in which digestive fluids were added, mechanical treatment of samples, and the pH at which all stages occurred. Water was added to simulate the salivary portion of digestion in lieu of alpha-amylase as preliminary studies found no significant difference in the antioxidant level available from samples treated with water compared to those treated with alpha-amylase (data unpublished, Moore 2006). Samples were kept in the dark under nitrogen at ambient temperature for analysis.

	Gastric fluid	Duodenal fluid	Bile fluid
Inorganic solutions	15.7 mL 2.99 M NaCl 3 mL 740.12 mM NaH <sub>2</sub> PO <sub>4</sub> 9.2 mL 1.20 M KCl 18 mL 151.04 mM CaCl <sub>2</sub> -2H <sub>2</sub> O 10 mL 572.07 mM NH <sub>4</sub> Cl 6.5 mL 1.02 M HCl	40 mL 2.99 M NaCl 40 mL 1.01M NaHCO <sub>3</sub> 10 mL 58.78 mM KH <sub>2</sub> PO <sub>4</sub> 6.3 mL 1.20 M KCl 10 mL 52.53 mM MgCl <sub>2</sub> -6H <sub>2</sub> O 0.18 mL 1.02 M HCl	15 mL 2.99 M NaCl 34.15 mL 1.01M NaHCO <sub>3</sub> 2.1 mL 1.20 M KCl 0.075 mL 1.02 M HCl
Organic solutions	10 mL glucose 10 mL glucuronic acid 3.4 mL urea 10 mL glucosamine HCl	4 mL urea	5 mL urea
Enzymes & final solution add-ins	3200 mg pepsin	2500 mg pancreatin 9 mL CaCl <sub>2</sub> -H <sub>2</sub> O	15,200 mg 50% bile 5 mL CaCl <sub>2</sub> -H <sub>2</sub> O
Fluid pH	1.3 ± 0.02	8.1 ± 0.02	8.1 ± 0.02

**Table 4.1: Fed-state synthetic digestive fluid composition\***



**Figure 4.1 In-vitro digestion.**

### **Total phenolic content**

Total phenolic content of the digested pizza crust samples was measured using the Folin-Ciocalteu reagent according to a laboratory procedure (Yu et al. 2002). The final reaction mixture contained 100 µL of digested pizza crust, 500 µL of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate and 1.5 mL pure water. The absorbance at 765 nm was recorded after two hours incubation at ambient



temperature. A gallic acid standard was utilized to calculate the phenolic content. Duplicate measurements per digested pizza crust sample extract were taken.

### **Radical Cation ABTS•+ scavenging activity**

The 37.5% acetone extracts of pizza crust were tested against ABTS•+ which was generated according to a laboratory protocol (Miller, Rice-Evans 1997, Zhou and Yu 2004). Briefly, an ABTS•+ solution was prepared by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide 30 minutes at ambient temperature. The solution was then filtered and adjusted with phosphate buffer to an absorbance of 0.7 at a wavelength of 734 nm. A trolox standard was prepared with 37.5% acetone and the blank consisted of 1mL phosphate buffer and 80 µL water blank from the in-vitro digestion model. Likewise the final reaction mixture contained 1mL of the ABTS•+ solution, and 80 µL of the sample of trolox standard. The reaction mixture was vortexed 30 seconds and a reading recorded at 1.5 minute. Data is reported as trolox equivalents per gram of digested pizza crust as calculated using the trolox standard curve.

### **Oxygen radical absorbing capacity (ORAC)**

ORAC was conducted for 37.5% acetone extracts of the digested pizza crust samples using the fluorescent probe fluorescein (FL) according to laboratory protocol (Huang et al. 2002, Zhou and Yu 2004). Trolox standards and samples were prepared

in 37.5% acetone. AAPH and FL solutions were prepared with 75 mM phosphate buffer (pH 7.4). The final reaction mixture per well contained 225  $\mu$ L of 81.63 nM FL solution, 30  $\mu$ L blank (37.5% acetone), standard, or sample solution, and 25  $\mu$ L 0.36 M AAPH solution. A total volume of 280  $\mu$ L was constant for all wells. Standards and samples were run in duplicate simultaneously utilizing a Victor<sup>2</sup> multilabel counter (PerkinElmer, Turku, Finland). The reaction mixture was held at 37 °C and fluorescence measurements taken every minute at excitation and emission wavelengths of 485 nm and 530 nm respectively for 1.5 hours. Results were expressed as trolox equivalents (TE) calculated using the relative area under the curve for the samples as compared to the trolox standard curve run simultaneously. Samples of the same thermal treatment were combined and reported as the mean and the standard deviation.

### **Hydroxyl Radical Scavenging Capacity**

Hydroxyl radical scavenging capacity was measured by oxidation by the hydroxyl radical generated by ferric iron ( $\text{Fe}^{3+}$ ) and hydrogen peroxide and described previously (Moore 2006). Briefly, trolox standards were prepared in 37.5% acetone and pure acetone was used as the blank. Distilled water was used to prepare an 3.43 mM iron chloride ( $\text{FeCl}_3$ ) solution and a 0.1990 M  $\text{H}_2\text{O}_2$  solution. A 92.8  $\mu$ M solution of fluorescein (FL) was prepared using phosphate buffer. The final well concentration was 300  $\mu$ L per well consisting of 170  $\mu$ L FL solution, 30  $\mu$ L blank, standard, or sample, 40  $\mu$ L  $\text{H}_2\text{O}_2$  and 60  $\mu$ L  $\text{FeCl}_3$ . Degradation of the FL solution was measured every minute using a Victor<sup>2</sup> multilabel counter (PerkinElmer, Turku,

Finland). Trolox equivalents were calculated using the area under the curve as compared with the trolox standard run simultaneously with the samples. Data is reported as the mean of the thermal treatments and their standard deviations.

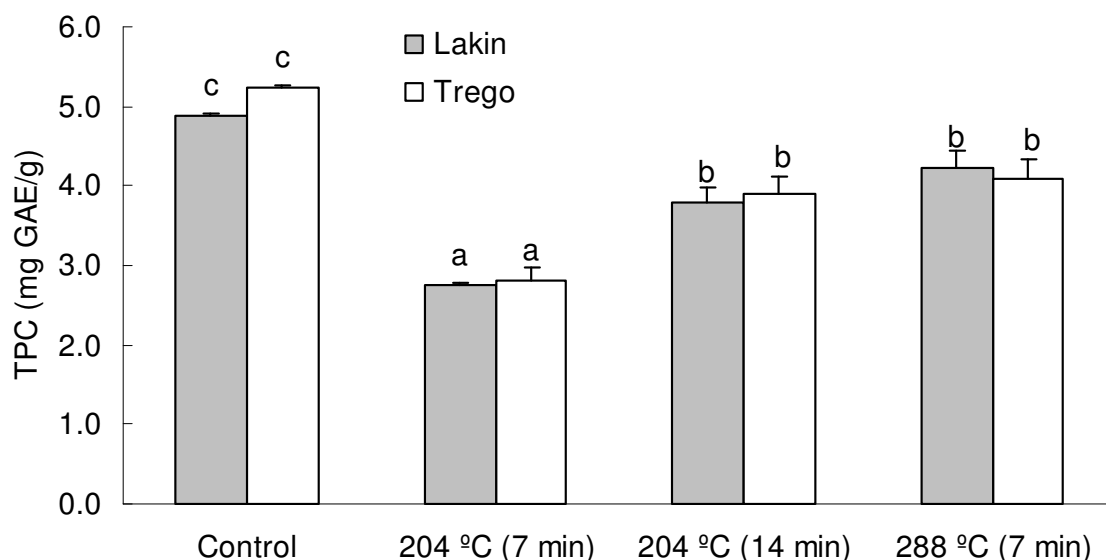
### **Statistical analysis**

Data were reported as mean and standard deviation for triplicate measurements. Analysis of variance and Tukeys honestly significant difference tests were conducted (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to determine differences among means. Statistical significance was declared at  $P < 0.5$ .

### **Results and Discussion**

#### **Total Phenolic Content**

In-vitro bioavailable total phenolic content (TPC) of pizza crusts are expressed as mg gallic acid equivalents (GAE) per gram of pizza crust. Baked pizza crust had significantly lower bioavailable TPC than the corresponding dough control, however extending baking time or increasing baking temperature increased in in-vitro bioavailable TPC (**Figure 4.2**). Previous TPC analysis of 50% acetone extracts of baked whole wheat pizza crusts showed increased TPC values as thermal treatment increased (Moore et al. 2007).

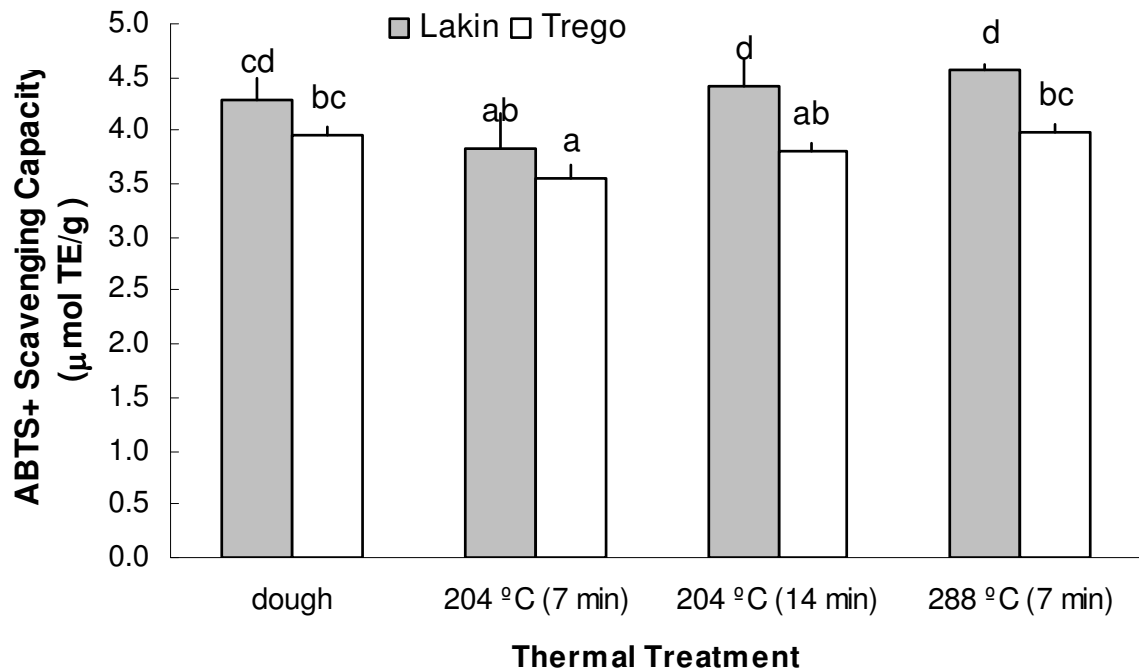


**Figure 4.2. In-vitro bioavailable total phenolic contents (TPC) of baked pizza crusts.** Results are expressed as milligrams gallic acid equivalents (GAE) per gram of sample on a dry weight basis. Controls were unbaked pizza dough samples. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. All measurements were conducted in duplicate and triplicate values of thermal treatment combined to report the mean ( $n = 6$ ) and standard deviation. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

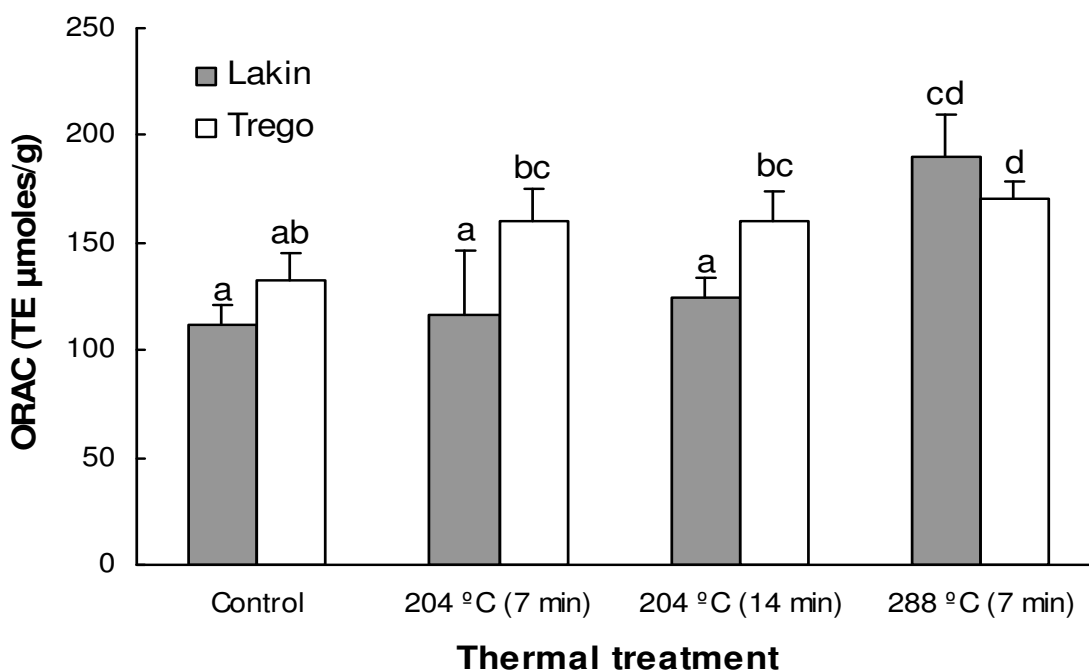
#### Radical Cation ABTS•+ scavenging activity

ABTS•+ scavenging activity ranged from 3.55 to 4.55  $\mu$ moles trolox equivalents (TE) per gram of pizza crust (**Figure 4.3**). The greatest ABTS•+ scavenging activity was observed in the in-vitro digested pizza crust prepared with Lakin wheat at the increased thermal treatment of 288 °C for 7 minutes and 204 °C for 14 minutes. The ABTS•+ scavenging activity was statistically different between the two varieties, however the trend for the Trego variety was the same in that ABTS•+

scavenging activity increased with thermal treatment. This finding is supported by previous data that found different varieties of Maryland grown soft wheat to have significantly different ABTS•+ scavenging activity (Moore et al. 2005).



**Figure 4.3 In-vitro bioavailable ABTS•+ scavenging capacities of baked pizza crust.** Results are expressed as  $\mu$ moles trolox equivalents (TE) per gram of sample on a dry weight basis. Controls were unbaked pizza dough samples. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. All measurements were conducted in duplicate and triplicate values of thermal treatment combined to report the mean ( $n = 6$ ) and standard deviation. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).



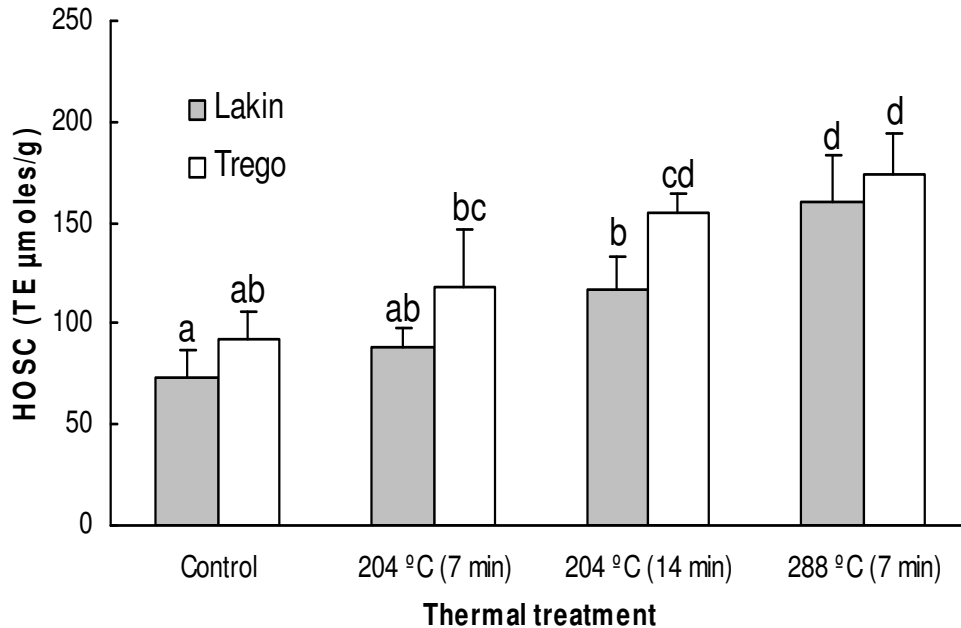
**Figure 4.4 In-vitro bioavailable ORAC scavenging capacities of baked pizza crust.** Results are expressed as  $\mu\text{moles trolox equivalents (TE)}$  per gram of sample on a dry weight basis. Controls were unbaked pizza dough samples. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. All measurements were conducted in duplicate and triplicate values of thermal treatment combined to report the mean ( $n = 6$ ) and standard deviation. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

### Oxygen radical absorption capacity (ORAC)

The ORAC assay measured the capacity of in-vitro digested pizza crust samples against the peroxyl radical. Samples with higher ORAC values may exert a protective effect for biomolecules against free radicals by donating a hydrogen atom. ORAC values are expressed as  $\mu\text{moles trolox equivalents (TE)}$  per gram of pizza crust (**Figure 4.4**). ORAC values were significantly higher at 190.0 and 170.8

$\mu\text{moles TE/g}$  for digested pizza crusts that had been baked at 288 °C for 7 minutes for Lakin and Trego varieties, respectively than for other treatments. It is significant to note that the ORAC for all digested, baked samples is greater than their corresponding dough samples with ORAC values of 112.2 and 132.6  $\mu\text{moles TE/g}$  for Lakin and Trego, respectively.

#### Hydroxyl radical scavenging capacity (HOSC) values



**Figure 4.5 In-vitro bioavailable HOSC scavenging capacity of antioxidants from whole wheat pizza crust.** Results are expressed as  $\mu\text{moles trolox equivalents (TE)}$  per gram of sample on a dry weight basis. Controls were unbaked pizza dough samples. Baking time and temperature combinations consisted of 204 °C for 7 minutes, 204 °C for 14 minutes, and 288 °C for 7 minutes. All measurements were conducted in duplicate and triplicate values of thermal treatment combined to report the mean ( $n = 6$ ) and standard deviation. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ( $P < 0.05$ ).

Hydroxyl radical scavenging capacity values for the digested pizza crust samples are reported as  $\mu\text{moles trolox equivalents/g}$  digested pizza crust on a dry weight basis (**Figure 4.5**). Results corresponded with ORAC data as there were significant increases in HOSC as thermal treatment increased. The highest HOSC values were 160.6 and 174.0  $\mu\text{moles TE/g}$  for digested pizza crusts that had been baked at 288 °C for 7 minutes for Lakin and Trego varieties, respectively than for other treatments. The lowest, yet significant HOSC values were the dough samples at 77.4 and 97.3  $\mu\text{moles TE/g}$  for the Lakin and Trego varieties, respectively.

### Conclusion

Results from this study demonstrate for the first time that in-vitro bioavailability of wheat antioxidants is increased when baking time or temperature was increased in baked whole wheat pizza crust. Results indicate that a food with higher available antioxidants may have a greater level of bio-available antioxidants. More research is required to determine if in-vitro methods for antioxidant analysis are correlated in-vivo measures such as a decrease in blood oxidative status. In addition, correlating the extractable components with bioavailability may lead to economic means of analysis to predict the health benefits from antioxidants in food systems.

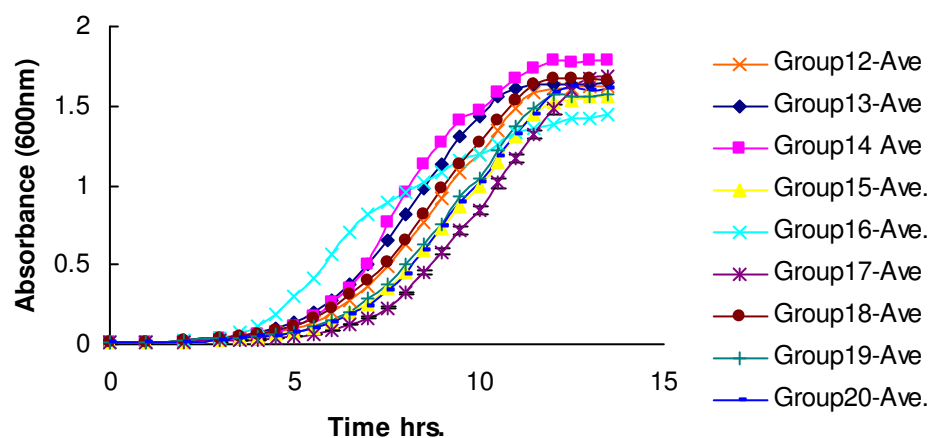


### Literature Cited

- Adom, K., Sorrells, M., Liu, R. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *J. Agric. Food Chem.* **2005**, 53, 2297-2306.
- Cheng, Z.; Su, L.; Moore, J.; Zhou, K.; Luther, M.; Yin, J.; Yu, L. Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *J. Agric. Food Chem.* **2006**, 54, 5623-5629.
- Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**, 50, 1815-1821.
- Kern, S.M.; Bennet, R.N.; Mellon, F.A.; Kroon, P.A.; Garcia-Conesa, M.T. Absorption of hydroxycinnamates in humans after high-bran cereal consumption. *J. Agric. Food Chem.* **2003**, 51, 6050-6055.
- Krygier, K. Sosulski, F., Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, 30, 330-334.
- Leenhardt, F.; Lyan, B.; Rock, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesy, C. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *J. Agric. Food Chem.* **2006**, 54, 1710-1715.
- Liyana-pathirana, C. Shahidi, F. Antioxidant activity of wheat extracts as affected by *in vitro* digestion. *Biofactors*. **2004**, 21, 325-328.
- Moore, J.; Hao, J.; Zhou, K.; Luther, M.; Costa, J.; Yu L. Carotenoid, tocopherols, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, 53(17), 6649-6657.
- Moore, J.; Cheng, Z.; Hao, J.; Guo, G.; Liu, J.-G.; Lin, C.; Yu, L. Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *J. Agric. Food Chem.* **2007**, 55, 10173-10182.
- Moore, J.; Cheng, Z.; Su, L.; Yu, L. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *J. Agric. Food Chem.* **2006**, 54, 9032-9045.
- Moore, J.; Luther, M.; Cheng, Z.; Yu, L. Effects of baking conditions, dough fermentation, and bran particle size on antioxidant properties of whole-wheat pizza crust. *J. Agric Food Chem.* **2008**. In submission.

- Miller, N.J., Rice-Evans, C.A. Factors influencing the antioxidant activity determined by the ABTS+ radical cation assay. *Free Radical Research*. **1997**. 26, 195-199.
- Oomen, A.G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; De Wiele, T.V.; Wragg, J.; Rompelberg, C. J.M.; Sips, A.-J.A.M.; Van Wijnen, J. H. Comparison of five in-vitro digestion models to study bioaccessibility of soil contaminants. *Environ. Sci. Tech.* **2002**. 36, 3326-3334.
- Price, R.K., Welch, R.W., Lee-Manion, A.M., Bradbury, I., Strain, J.J. Total phenoli and antioxidant potential in plasma and urine of humans after consumption of wheat bran. *Cereal Chem.* **2008**. 85(2), 152-157.
- Ross, A. Kamal-Eldin, A., Aman, P. Dietary alkylresorcinols: Absorption, bioactive, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutrition Reviews*. **2004**, 81-95.
- Slavin, J. Why whole grains are protective: biological mechanisms. *Proceedings of the Nutr. Society*. **2003**, 62, 129-134.
- USDA. Economic Research Services. (2006). Wheat: Market Outlook. Accessed August 12, 2008. <http://www.ers.usda.gov/briefing/Wheat/2005baseline.htm>
- Versantvoort, C.H.M.; Oomen, A.G.; Van de Kamp, E.; Rompelberg, C.J.M.; Sips, A.J.A.M. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chem. Tox.* **2005**. 43(1), 31-40.
- Yu, L.; Haley, S.; Perret, J.; Harris, M. Antioxidant properties of hard winter wheat extracts. *Food Chem.* **2002**, 78, 457-461.
- Yu, L.; Zhou, K. Antioxidant properties of bran extracts from 'Platte' wheat grown at different locations. *Food Chem.* **2004**, 90, 311-16.
- Zhou, K.; Yin, J.; Su, L.; Haley, S.; Yu, L. Phenolic acid, tocopherols, and carotenoids compositions, and antioxidant functions of hard red winter wheat bran. *J. Agric. Food Chem.* **2005**. 53, 3916-3922.
- Zhou, K.; Laux, L.; Yu, L. Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *J. Agric. Food Chem.* **2005**. 52, 1118-1123.
- Zhou, K. Yu, L. 2004. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem.* **2004**, 52, 1112-1117.

## APPENDIX A. Yeast Log Growth Curve\*



\* Log growth curves were conducted for all yeasts. Yeasts were later presumed to be in logarithmic growth between 6 and 10 hrs. following the same pre-treatment conditions.

## Chapter 5: Development of a Tri-fold to Increase Whole Wheat Pizza Crust Consumption in College Students

### Abstract

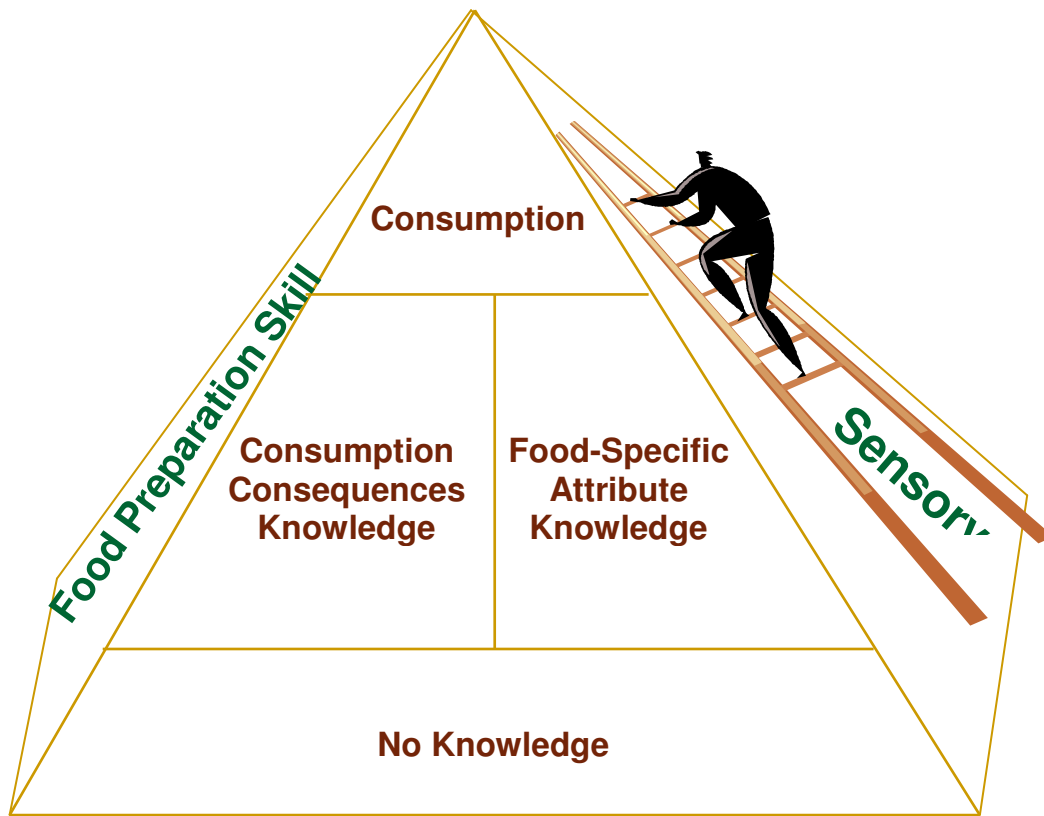
Print materials are an effective means of distributing information. A tri-fold was developed to promote the preparation and consumption of whole wheat pizza among college students. Tri-fold information sheets including a whole wheat pizza dough recipe, three healthy recipe topping sets with nutritional information, and relevant health claims and nutritive definitions were developed. Coupling health claims with popular foods items may be an effective way to increase nutritional quality of collegiate diets.

### Introduction

The United States and other developed nations are recording alarming trends in obesity with cardiovascular disease and cancer remaining the top two causes of mortality. The American Dietetic Association states that the “total diet or overall pattern of food eaten is the most important focus of a healthful eating style (Nitzke & Freeland-Graves 2007).” Healthful recipes and directions can help overcome barriers to preparing meals at home such as poor cooking skills, time constraints, cost, and available ingredients (Cunningham 2007). Hands on cooking activities have successfully increased cooking skills, confidence, and eating habits of preschool

children and their families, college students, and WIC participants (Bensley et al. 2006, Condrasky et al. 2006, Dougherty & Silver 2007, Michaud et al. 2007).

Adolescents and young adults transitioning from parental supervision to independent living are developing their eating behaviors and dietary practices which will affect their health (Larson et al. 2007, Richards et al. 2006). A study of young adults found that young adults that purchased their own food and prepared meals at home had improved diet quality over their counterparts who did so less frequently (Larson et al 2006a, Larson et al 2006b). Targeting nutrition education at young adults and college students is particularly important as 19-34 yr. olds are less knowledgeable about diet-cancer connections, National Cancer Institute dietary recommendations, and composition of foods (Georgiou et al. 1997).



**Figure 5.1 Hierarchy of knowledge, psychomotor skill, and food related sensory attributes leading to increased consumption of functional foods.** Knowledge related attributes based upon the hierarchy of nutritional knowledge by Brian Wansink (Wansink et al. 2005).

This addresses the critical issues, “People do not know foods to eat for a balanced diet (Kim et al 2006).” Effective public health practice is dependent upon scientists and other health professional’s ability to appropriately communicate to diverse audiences, the majority of which are individuals outside the scientific community (Nelson 2002). **Figure 5.1** illustrates the belief that when multiple barriers are overcome such as nutritional knowledge, food preparation ability, and sensory preferences, functional foods are more likely to be consumed. Materials that

allow participants to be interactive, prepare pizzas' at home, may encourage more skill development and behavior change than cognitive knowledge alone.

#### Tri-fold Development

Tri-folds were developed for easy print material distribution to encourage the production and consumption of whole wheat pizza (**Figures 5.2 & 5.3**). Tri-folds were tested in house for general appearance acceptability and readability. Information included on the tri-fold was: whole wheat pizza crust recipe and directions, three recipe sets for final pizza products, nutrition facts, whole wheat health claims and suggested intake, and findings from our laboratory to promote food preparation practices that maintain or increase antioxidant availability.



### Functional Whole Wheat Pizza Crust – High Antioxidant Recipes Included!

- **Whole wheat** flour is naturally rich in antioxidants
- Increasing dough **fermentation** time may increase some antioxidant properties
- Increasing pizza crust baking **time** or **temperature** may increase its antioxidant properties



Laboratory of Nutraceutical & Functional Food Development

#### Thin Crust Functional Whole Wheat Pizza Crust Recipe

**Ingredients:**

**1 packet** active dry yeast (2 ¼ teaspoons)  
**1 cup** warm water (100-110°F)  
**3 tablespoons** honey  
**1 tablespoon** plus **1 teaspoon** vegetable oil  
**1 ¼ teaspoons** salt  
**2 ¾ cups** whole wheat flour

**The day before...**

- 1) Dissolve yeast in warm water in a small bowl.
- 2) Add honey, oil and salt. Stir with whisk until honey and salt dissolve.
- 3) Measure flour into large mixing bowl and slowly add water mixture to the flour mixing until dough forms.
- 4) Transfer dough to floured surface and knead by hand for 2 minutes. Dough may be sticky.
- 5) Divide dough into 3 sections and shape each section into a ball
- 6) Transfer dough balls to a lightly oiled pan, leaving a few inches for each ball to raise.
- 7) Cover pan loosely with plastic wrap.
- 8) Store pan overnight (~18 hours) in the refrigerator.

**The day of...**

- 1) Allow dough to come to room temperature.
- 2) Preheat oven to 500°F.
- 3) Roll each ball out to a thin 12" circle and place on a pizza screen.
- 4) Top with tomato sauce, vegetables, and desired toppings.
- 5) Put pizza in middle rack of preheated oven. Bake for 6-8 minutes, until crust gets browned.  
 (A pizza pan or stone can also be used to bake the pizzas. Baking times may change.)

#### Sweet & Savory Slices

**Ingredients:**

1 12" Pizza Crust  
 ½ cup pizza sauce  
 ½ cup sliced bell pepper  
 ¼ cup chopped Canadian bacon  
 ½ cup diced pineapple  
 ⅓ cup low fat mozzarella cheese, shredded  
 ⅓ cup provolone cheeses, pieces

**Directions:**

- 1) Spread pizza sauce over crust
- 2) Sprinkle with bell pepper, bacon, and pineapple
- 3) Top with provolone and mozzarella cheeses
- 4) Bake at 500°F for 6-8 minutes

**Nutrition Facts:** Serving Size: 1/4 pizza, Calories: 250, Total Fat: 7g, Cholesterol: 15mg, Sodium 660mg, Total Carbohydrates: 36g, Dietary Fiber: 5g, Sugars: 10g, Protein 12g



**Figure 5.2 Functional Whole Wheat Pizza Tri-fold – Side A**



### Antioxidants:

“A dietary antioxidant is a substance that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans.” - IOM

### Wheat & Health:

“diets rich in whole grain foods and other plant foods, and low in saturated fats and cholesterol, may reduce the risk of heart disease and certain cancers” – FDA

“Consuming 3 or more 1-oz equivalent servings of whole grains/day can reduce risk of several chronic diseases and may with weight management” – USDA

### Functional Foods:

“Foods or dietary components that may provide a health benefit beyond basic nutrition.”

### Creamy Pesto Pizza

#### Ingredients:

- 1 12” Pizza Crust
- ½ cup pesto sauce
- ¾ cup torn spinach leaves
- ¼ cup low-fat ricotta cheese
- ¼ cup feta cheese, crumbled

#### Directions:

1. Spread pesto evenly over crust
2. Sprinkle with spinach leaves
3. Use two spoons to drop small balls of ricotta onto the pizza
4. Crumble feta cheese over the top
5. Bake at 500°F for 6-8 minutes

**Nutrition Facts:** Serving Size: 1/4 pizza, Calories: 300 Total Fat 15g, Cholesterol 7mg, Sodium 600mg, Total Carbohydrate 36g, Dietary Fiber 6g, Protein 13g

### Herb Garden Fresh Pizza

#### Ingredients:

- 1 12” Pizza Crust
- 1 cup Diced Tomatoes
- ¼ cup sliced bell pepper
- ¼ cup chopped red onion
- ½ cup sliced mushrooms
- ¼ cup sliced olives
- ¼ cup chopped basil
- ½ cup low fat mozzarella
- 1 slice American cheese

#### Directions:

1. Spread diced tomatoes over crust
2. Sprinkle with vegetables and basil
3. Tear American cheese into pieces and sprinkle over toppings
4. Top with shredded mozzarella
4. Bake at 500°F for 6-8 minutes

**Nutrition Facts:** Serving Size: 1/4 pizza, Calories: 220, Total Fat 5g, Cholesterol 10mg, Sodium 420mg, Total Carbohydrate 36g, Dietary Fiber 7g, Protein 10g



For more information contact:  
Marla W. Luther [mluther@umd.edu](mailto:mluther@umd.edu)  
Margaret Slavin, R.D. [mms@umd.edu](mailto:mms@umd.edu)



Funding for this research provided  
by USDA-CSREES/NRI

Figure 5.3 Functional Whole Wheat Pizza Tri-fold – Side B

## Pizza Recipes

Pizza recipes were developed in the Laboratory of Nutraceuticals & Functional Foods at the University of Maryland, College Park. Suggested pizza dough fermentation and baking times were selected based on our tests to analyze the effects of processing on the antioxidant levels of whole wheat pizza crust. The effects of baking time and temperature on sensory products were also tested in-house to ensure that quality of the final product. Three pizza recipes: Sweet & Savory Slices (SS), Herb Garden Fresh Pizza (HG), and Creamy Pesto Pizza (CP) were developed and tested in-house for quality. Nutritional facts were generated using Diet Analysis Plus 6.0 (Salem, OR) and reported on a per serving basis. The goal of the variety of these recipes was to: provide recipes for which there was a degree of familiarity among college students (SS), provide a vegetable based healthier option (HG), and suggest alternative sauces to those who may have diet restrictions (CP).

## Health Claims

Health claims are intended to, “benefit consumers by providing information on healthful eating patterns that may help reduce the risk of heart disease, cancer, osteoporosis, high blood pressure, dental cavities, or certain birth defects (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005).” The nutritional impact of inclusions of health claims on products is not well established. Consumers generally perceive health claims as helpful, are more likely to purchase items with health

claims, and may make healthier dietary choices, however the accuracy and truthfulness of health and nutrition claims is widely questioned amongst all populations (Marietta 1999, Williams 2005, Misra 2007). Nonetheless, health claims were provided in the tri-fold to provide readers with food-specific attribute knowledge and consumption consequences knowledge.

### Conclusion

Outreach materials containing both consumption consequence knowledge (health claims) and food-specific attribute knowledge (antioxidants) may increase consumption over outreach materials with only one form of knowledge. Simple recipes and directions for commonly consumed foods may increase at-home food preparation which has been correlated with increased nutritive value of foods. This research indicates that creating outreach materials for functional forms of commonly consumed items may increase their adaptation. More research is required to document the effect of recipe outreach methods for college students.

### Literature Cited

- Arvanitoyannis, I.S; Houwelingen-Koukaliaroglou, H.S. Functional Foods: A survey of health claims, pros and cons, and current legislation. *Crit. Review Food Sci.* **2005**. 43, 385-404.
- Bensley, R.J.; Brusk, J.J.; Anderson, J.A.; Mercer, N.; Rivas, J.; Broadbent, L.N. wichealth.org: Impact of a stages of change-based internet nutrition education program. *J Nutr Educ Behav*. **2006**. 38, 222-229.
- Condasky, M.; Graham, K.; Kamp, J. Cooking with a Chef: An innovative program to improve mealtime practices and eating behaviors of caregivers of preschool children. *J Nutr Educ Behav*. **2006**. 324-325.
- Cunningham, E. Where can I find resources to assist my client with meal planning? *J. Amer. Diet. Assoc.* **2007**. 10, 1068.
- Dougherty, K.; Silver, C. Chef-Nutritionist team spark enjoyment and learning in cooking education series for 8- to 12-year olds. *J Nutr Educ Behav*. **2007**. 39, 237-238.
- Georgiou, C.C.; Betts, N.M.; Hoerr, S.I.; Keim, K.; Peters, P.K.; Stewart, B.; Voichick, J. Among young adults, college students and graduates practiced more healthful habits and made more healthful food choices than did nonstudents. *J Am Diet Assoc.* **1997**. 97, 754-759.
- Kim, E.; Koh, B., Ng, J.; Su, R. myPyramid: Increasing nutritional awareness. *CHI Extended Abstracts*. **2006**, 1843-1848.
- Larson, N.I.; Perry, C.L.; Story, M.; Neumark-Sztainer D. Food preparation by young adults is associated with better diet quality. *J. Amer. Diet. Assoc.* **2006a**. 106, 2001-2007.
- Larson, N.I.; Story, M.; Eisenberg, M.E.; Neumark-Sztainer D. Food preparation and purchasing roles among adolescents: Associations with sociodemographic characteristics and diet quality. *J. Am Diet. Assoc.* **2006b**. 106, 211-216.
- Larson, N.I.; Neumark-Sztainer, D.; Hannan, P.J.; Story, M. Family meals during adolescence are associated with higher diet quality and healthful meal patterns during young adulthood. *J Am Diet Assoc.* **2007**. 107, 1502-1510.
- Marietta, A.B.; Welshimer, K.J.; Anderson, S.L. Knowledge, attitudes, and behaviors of college students regarding the 1990 Nutrition Labeling Education Act food labels. *J. Amer. Diet Assoc.* **1999**. 99, 445-449.

- Michaud, P.; Condrasky, M.; Griffin, S.F. Review and application of current literature related to culinary programs for nutrition educators. *Top Clin Nutr.* **2007.** 22, 336-348.
- Misra, R. Knowledge, attitudes, and label use among college students. *J. Amer. Diet Assoc.* **2007.** 107, 2130-2134.
- Nelson, D.E. Current Issues and Challenges. In. "Communicating Public Health Information Effectively, A Guide for Practitioners." Nelson, D.E.; Brownson, R.C.; Remington, P.L.; Parvanta, C. American Public Health Association. Washington, DC. **2002,** 1-10.
- Nitzke, S. and Freeland-Graves, J. Position of the American Dietetic Association: Total diet approach to communicating food and nutrition information. *J. Amer. Diet. Assoc.* 2007. 107, 1224-1232.
- Richards, A.; Kattelman, K.K.; Ren, C. Motivating 18- to 24-year olds to increase their fruit and vegetable consumption. *J Am Diet Assoc.* **2006.** 106, 1405-1411.
- Wansink, B.; Westgreen, R.E.; Cheney, M.M. Hierarchy of nutritional knowledge that relates to the consumption of a functional food. *Nutrition.* **2005.** 21, 264-268.
- Williams, P. Consumer understanding and use of health claims for foods. *Nutrition Reviews.* **2005.** 62(7), 256-264.